Egg Consumption and Risk of Cardiovascular Disease and Type 2 Diabetes

EGG CONSUMPTION AND RISK OF CARDIOVASCULAR DISEASE AND TYPE 2 DIABETES

AMANDA LEE PROUTY SANDS

A Dissertation Submitted to the Faculty of

The Harvard T.H. Chan School of Public Health

in Partial Fulfillment of the Requirements

for the Degree of Doctor of Science

in the Department of Nutrition

Harvard University

Boston, Massachusetts.

November, 2015

Dissertation Advisor: Dr. Frank Hu Amanda Lee Prouty Sands

Egg Consumption and Risk of Cardiovascular Disease and Type 2 Diabetes

ABSTRACT

Due to their cholesterol content, limiting egg intake has been widely recommended for prevention of cardiovascular disease (CVD). However, recent reports by the 2015 Dietary Guidelines Committee and AHA/ACC suggest that there is insufficient evidence that dietary cholesterol is appreciably associated with blood cholesterol. In addition, the literature on the association with type 2 diabetes (T2D) is inconsistent. These analyses aim to determine the association between egg intake and the risk of CVD and T2D in the Nurses' Health Study (NHS), the Nurses' Health II Study (NHSII), Health Professionals Follow-up Study (HPFS) and the NIH-AARP Diet and Health Study (AARP), and conduct a meta-analyses.

Egg intake was assessed via validated semi-quantitative food frequency questionnaires. Cox proportional hazard models, adjusted for age, lifestyle and dietary factors, were used to estimate relative risks (HR) and 95% Confidence Intervals (CI). We observed 12,832 and 16,570 cases of incident CVD and T2D in NHS, NHSII and HPFS, and 11,268 CVD mortality cases in AARP. An increase of one egg per day was not associated with risk of CVD in NHS, NHSII and HPFS (HR: 1.04, 95% CI: (0.96, 1.13)). In the AARP study an increase of one egg per day was associated with an increased risk of CVD mortality (HR: 1.13, 95% CI: (1.05, 1.20)) and, an increased risk of CVD mortality among diabetics (HR: 1.25, 95%CI: (1.11, 1.41)). One egg per day was associated with an increased risk of CVD (HR: 1.05, 95% CI: (1.01, 1.09)) in a meta-analysis of the current results and previously published studies. We also saw an increased risk of CVD among diabetics (HR: 1.24, 95% CI: (1.12, 1.37)). We observed an increased risk of T2D with an increase of one egg per day (HR: 1.09, 95% CI: (1.01, 1.18)) in NHS, NHSII and HPFS, and in the meta-analysis (HR: 1.13, 95% CI: (1.07, 1.19)).

Although there does not seem to be a significant association between egg intake and risk of CVD or T2D in healthy individuals, people at risk for CVD or T2D and those who currently have T2D may want to limit egg intake.

TABLE of CONTENTS

LIST of FIGURES

LIST of TABLES

CHAPTER 1

CHAPTER 2

CHAPTER 3

ACKNOWLEDGEMENTS

I would like to thank my advisor Frank Hu for his guidance both in and out of the classroom. I am very grateful for the opportunity to learn from him, and would not have been able to undertake this thesis without his advice, encouragement and support. I would also like to thank my committee members Meir Stampfer, Frank Sacks and Bernard Rosner who offered excellent feedback and numerous learning opportunities throughout the thesis process.

Thank you to Yanping Li, who was an immense help with my many questions and late-night emails about coding issues and statistical methods. Without your help, I would have been completely lost many times. DeeDee Tobias and Mike Pan, thank you for helping me get started and teaching me everything I needed to know about programming.

Thank you to my friends for offering both emotional and statistical support. You each made coming to school enjoyable even during the most stressful times. Thanks to my family who have supported all of my decisions, small or big, and have been an unwavering source of support throughout the process. Thank you to Zack for always encouraging me to continue learning and improving, and for being there to comfort me during the frustrations and to celebrate my achievements.

INTRODUCTION

Obesity and related chronic diseases continue to be a problem on the global scale. Specifically, Cardiovascular Disease (CVD) and Type 2 Diabetes (T2D) are two diseases that cause substantial burden worldwide. There are many modifiable lifestyle factors that play a role in the development of both CVD and T2D, including overweight and obesity, physical activity, smoking status, alcohol consumption and diet[1, 2]. In the past, limiting dietary cholesterol intake to 300mg/day was widely recommended for prevention of CVD[1]. More recently, the 2015 Dietary Guidelines Advisory Report did not carry forward the upper limit for dietary cholesterol due to limited evidence to support that dietary cholesterol has an appreciable relationship with blood cholesterol levels[3]. Eggs are a major source of dietary cholesterol and contain about 200mg, but they also contain a variety of other nutrients beneficial to health such as unsaturated fats, amino acids, and B-vitamins[4]. Previous research has found that consumption of fats such as polyunsaturated fatty acids may reduce risk of both CVD and T2D, while consumption of saturated and trans fats may increase risk[5, 6]. Although a number of studies have been conducted to examine the relationship between egg intake and the risk of CVD and T2D, the debate remains unresolved. The current research focuses on the association between whole egg intake and the risk of T2D and CVD.

In chapter 1, I examine the association between whole egg consumption and the risk of CVD in three prospective cohorts, the Nurses' Health Study (NHS), Nurses' Health Study II (NHSII) and Health Professionals Follow-up Study (HPFS). Although several prospective cohorts have not found any association between egg intake and the risk of CVD, other study designs such as cross-sectional analyses have found conflicting results, sparking debate in the literature[4, 7-14]. In the current analyses I assess egg intake of one or more eggs per day and the risk of overall CVD and of stroke and coronary heart disease (CHD) separately. I also examine the differences in the effect of eggs on CVD stratified by several CVD risk factors.

In chapter 2, I assess the association between whole egg consumption and the risk of CVD mortality in the NIH-AARP Diet and Health Study (AARP). Previous publications of CVD mortality have found similar results to those reporting incident CVD[9, 12, 13]. No previous studies have been able to

examine this relationship in an older population at higher risk for CVD. In the current analyses I examine the relationship between egg intake and the risk of CVD mortality as well as the overall association of one egg per day with the risk of total CVD through meta-analysis of currently published prospective cohorts.

In chapter 3, I examine the association of whole egg consumption with the risk of T2D in the NHS, NHSII and HPFS cohorts. Less is known about the association between eggs and risk of T2D. While several studies have found an increased risk of CVD among participants with T2D, the literature on the risk of incident T2D with egg consumption is inconsistent[15-21]. It is possible that increased dietary cholesterol may lead to inflammation processes and beta-cell dysfunction that put patients at higher risk for development of T2D[22, 23]. In the current analyses I assess the association of egg consumption of one egg or more per day with the risk of incident T2D. I also combine previous reports on the subject to determine an overall risk of T2D with an increase of one egg per day using meta-analysis.

BODY of DISSERTATION

CHAPTER 1

Egg Consumption and Risk of Cardiovascular Disease: Findings from Three Large Prospective US Cohort Studies of Men and Women

Amanda L. Sands, BS[1]; Yanping Li, MD, PhD[1]; Meir J. Stampfer, MD, DrPH[1,2,3]; Frank M. Sacks, MD[1,3]; Bernard Rosner, PhD[1,3,4]; Walter C. Willett, MD, DrPH[1,2,3]; Frank B. Hu, MD, PhD[1,2,3]

[1]Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, MA

[2]Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA

[3]Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA

[4]Department of Biostatistics, Harvard T.H. Chan School of Public Health, Boston, MA

ABSTRACT

Background

Because eggs contain high amounts of dietary cholesterol, limiting their consumption has been widely recommended for prevention of cardiovascular disease (CVD). We prospectively evaluated the association between egg intake and CVD risk in three large cohorts.

Methods and Results

The study population includes women from the Nurses' Health Study (1980-2010; N=69,387), Nurses' Health Study II (1991-2011; N=90,750), and men from the Health Professionals Follow-up Study (1986-2010; N=43,203); all were free of diagnosed CVD, type 2 diabetes (T2D), and cancer at baseline. Egg intake was assessed every 4 years via validated food frequency questionnaires. Incident coronary heart disease (CHD) (nonfatal myocardial infarction (MI) and fatal CHD) and stroke cases were ascertained via medical record reviews. Relative risks (HR) and 95% confidence intervals (CI) were estimated using multivariate Cox proportional hazards models, adjusted for age, lifestyle and dietary factors. We identified 12,832 incident cases of CVD. Overall, egg consumption up to 1 egg/day was not significantly associated with risk of incident total CVD after multivariate adjustment (HR (CI) by category: <1/month: 1(ref); 1-3/month: 1.02 (0.95, 1.09); 1/week: 1.01 (0.94, 1.08); 2-4/week: 0.97 (0.91, 1.04); 5-6/week: 1.05 (0.93, 1.18); 1+/day: 1.10(0.98, 1.23)) (p-trend=0.33). Egg intake was not associated with either CHD or stroke individually (p-trend: CHD=0.41, stroke=0.48). We estimated that replacing one egg with one serving of red and processed meats per day was associated with a 9% increased risk of CVD, while replacing eggs with low-fat dairy, nuts, legumes or whole grains was associated with a 9-11% lower risk of CVD.

Conclusions

Egg intake was not associated with increased risk of total CVD, stroke or CHD in these three prospective cohorts. However, replacing eggs with low-fat dairy, whole grains, legumes and nuts was associated lower CVD risk, while replacing eggs with red and processed meats was associated with increased risk.

INTRODUCTION

In the US, cardiovascular disease (CVD) is the leading cause of death in both men and women [24]. Many modifiable lifestyle factors play a role in the development of CVD, including overweight and obesity, physical activity, smoking status, alcohol consumption and diet [1]. In the past, limiting dietary cholesterol intake to 300mg/day has been widely recommended for prevention of CVD [1]. However, due to the lack of effect of dietary cholesterol on blood cholesterol, the 2015 Dietary Guidelines Advisory Committee Report did not carry forward the upper limit for dietary cholesterol [3]. Eggs are a major source of dietary cholesterol, and one egg contains about 200mg. A meta-analysis of feeding studies found that ingestion of dietary cholesterol had only modest effects on LDL and HDL cholesterol and little influence on the ratio of LDL to HDL[25]. Other research on dietary cholesterol and changes in CVD risk factors suggests that dietary cholesterol may increase LDL particle size, the number of large HDL particles, activity of Lecithin-cholesterol acyltransferase and Cholesteryl ester transfer protein and enhance reverse cholesterol transport, but does not change the ratio of LDL to HDL particles [26]. In addition, although eggs do contain a significant amount of dietary cholesterol, other beneficial nutrients such as unsaturated fats and B-vitamins are also present, potentially offsetting any possible increased risk due to the cholesterol content [4].

Multiple publications over the past decade have fueled the debate of the association between egg intake and the risk of CVD. Although a cross-sectional study found an increase in carotid plaque area with increases in egg consumption[7], several prospective cohort studies of egg consumption have not found an association between egg intake and risk of coronary heart disease (CHD) [4, 8-13] or stroke [9-14] after adjusting for dietary and lifestyle factors. A recent meta-analysis found no association between egg intake up to one egg per day for either myocardial infarction (MI) or total stroke, but did find a significantly decreased risk of hemorrhagic stroke in those who consumed 1 or more eggs per day (HR: 0.75, 95% CI: (0.57, 0.99))[27].

The current study is an updated analyses of previous results published in JAMA in 1999[4]. In the previous publication, we identified 1,124 cases of CVD after 8 years of follow-up in HPFS and 14 years

of follow-up in NHS. The current analysis expands on the previous publication by including additional years of follow-up, more than ten times the number of cases and extends the analysis to the younger cohort of the Nurses' Health Study II. Specifically, in the current analyses, we have identified 12,827 cases of CVD after 24 years of follow-up in HPFS, 30 years in NHS and 20 years in NHSII. We also estimated the effects of substituting other foods (red and processed meats, low-fat dairy, nuts, and whole grains) for eggs on risk of CVD.

METHODS

NHS, NHSII and HPFS are prospective cohort studies conducted in the US that began in 1976, 1989 and 1986, respectively. NHS includes 121,701 female registered nurses between the ages of 30-55. NHSII includes 116,430 female registered nurses between the ages of 25-44. HPFS includes 51,529 male health professionals between the ages of 40-75. Participants in all three cohorts completed validated questionnaires with information on disease diagnosis, disease risk factors and lifestyle characteristics every two years. Dietary information was collected every four years and follow-up rates were over 90%[28-30]. For all cohorts, participants were excluded if they reported unrealistic intake on the FFQ (>70 items blank, <500 or >3500 kcals/day for women and <800 or >4200 kcals/day for men), or if they had been previously diagnosed with cancer, T2D or CVD. In addition, participants were excluded if egg intake or total calories were missing at baseline. The final sample included 69,387 participants in NHS, 90,750 participants in NHSII and 43,159 participants in HPFS. The study protocol was approved by the Institutional Review Board at the Brigham and Women's Hospital in Boston.

Assessment of Egg Intake

Egg intake was reported every four years beginning in 1980, 1991 and 1986 for NHS, NHSII and HPFS, using a validated semi-quantitative FFQ with 61 items in 1980 and 131 items in subsequent questionnaires[31]. Participants were asked how often on average they have consumed eggs in the past year. Reported intake includes whole eggs prepared separately from other foods. FFQs were validated against

four 7-day weighed diet records in 173 women. Correlation between the FFQ and weighed food records was good, with Pearson correlation coefficients, corrected for within-person variation, for specific food items on the FFQ ranging from 0.17 (spinach) to 0.94 (yogurt and beer)[32]. Specifically, the de-attenuated correlation between the FFQ and weighed food record for egg intake was 0.77[32]. A similar validation study was conducted in 127 men in HPFS (de-attenuated correlation coefficient =0.80)[33]. Egg intake was analyzed in 6 categories of intake as follows: 0-1 per month, 2-3 per month, 1 per week, 2-4 per week, 5-6 per week and ≥1 per day for primary analyses. An additional category of ≥2 per day was used in secondary analyses.

Assessment of Incident CVD

Incident non-fatal CVD was initially ascertained through self-report questionnaire every two years. Fatal outcomes were ascertained by reports from the family and systematic searches of the National Death Index. Physicians blinded to risk factor status of the participants confirmed diagnoses through medical record review. CVD includes diagnoses of nonfatal MI, fatal CHD and stroke. The World Health Organization criteria were used to diagnose MI [34]. The patient must have typical symptoms and elevated cardiac enzymes or electrocardiographic changes. Stroke was confirmed using criteria from the National Survey of Stroke which required a typical neurologic defect of rapid onset[35]. Stroke subtypes (ischemic and hemorrhagic) were determined by through medical record review by a neurologist using the Perth Community Stroke Study Criteria[36]. Ischemic stroke included thrombotic, embolic or unspecified non-hemorrhagic, while hemorrhagic stroke included subarachnoid and intraparenchymal hemorrhages. When medical records were not available but confirmation was provided through interview or letter, cases were considered probable. Analyses include both confirmed and probable cases of CVD. Analyses including only confirmed cases produced virtually identical results. Date of death was collected through review of medical records, the National Death Index, tumor registries and death certificates.

Statistical Methods

Follow-up began with the return of the first dietary questionnaire, in 1980, 1991 and 1986 for the NHSI, NHSII and HPFS cohorts, respectively. Person-time was included until diagnosis of CVD, death or the end of follow-up on June 30th, 2010 for NHS, June 30th 2011 for NHSII and January 31st, 2010 for HPFS. Hazard ratios and 95% confidence intervals were calculated using multivariate adjusted Cox proportional hazards analyses for incident CVD. We used a cumulative average update of dietary variables, including egg intake, to limit within-person variation and to represent long-term diet[37]. The proportional hazards assumption was tested through a likelihood ratio test of models with and without an egg intake and time-to event interaction term. A possible linear trend for the RR estimates across categories of egg consumption was evaluated using the median of each category of egg intake as the dose of egg consumption [38]. Total CVD was analyzed separately as total CHD (including non-fatal MI and fatal CHD) and total stroke.

Model 1 included adjustment for age, cohort, and two year time intervals. Model 2 was additionally adjusted for sex, race/ethnicity, BMI, smoking status, physical activity, family history of MI, baseline diagnosis with hypertension or high blood cholesterol, previous statin use, alcohol consumption, multivitamin use and for women; postmenopausal status, use of menopausal hormones, and use of oral contraceptives. Model 3 additionally included total energy intake and foods associated with egg intake (e.g. red meat, bacon, other processed meat, whole milk, refined carbohydrates, potatoes and French fries, juice, sugar-sweetened beverages and coffee). Adherence to the western dietary pattern was also considered as a possible confounder but not included in the final model because it did not significantly change hazard ratios or overall model fit. Stratified analyses and likelihood ratio tests for interactions were conducted for updated BMI, physical activity, self-reported hypertension (including use of anti-hypertensive medication), self-reported high blood cholesterol (including use of cholesterol lowering medication), family history of MI, statin use, smoking status, age and self-reported T2D. Stratified analysis of T2D includes participants reporting T2D at baseline.

In sensitivity analyses, the results for total stroke were further examined by ischemic and hemorrhagic stroke. We also examined results for coronary artery bypass graft (CABG). We examined the risk of CVD for egg intake of 2 or more eggs per day. In addition, we explored the risk of CVD for total egg intake (including eggs in baked goods). To determine whether a cumulative average update was the appropriate model for risk of CVD, we examined results from baseline diet only, which would be used with a long latency disease but does not minimize measurement error in dietary variables. We also examined results from a model using the most recent diet which would be useful for a shorter latency disease[37]. To account for the possibility that diagnosis with an intermediate endpoint of CVD could introduce confounding, we examined results where we stopped updating diet after diagnosis with T2D, hypertension, high blood cholesterol, angina, CABG or beginning statin use[39]. We examined the effects of substituting eggs for 1 serving of other common food groups on risk of total CVD by including continuous variables for both eggs and the other food in the model along with other non-dietary confounders. We then used the differences between the beta-coefficients, variance and the covariance of eggs and the other food to calculate HR's and 95% CI's[40]. All p-values are two-sided and statistical analyses were performed using SAS v.9.3 (SAS Institute, Inc., Cary, NC).

RESULTS

Through the end of follow-up, we identified 12,832 total cases of CVD (NHS: 5,698; NHSII: 998; HPFS: 6,145) including 4,805 cases of total stroke, 750 cases of hemorrhagic stroke, 2,533 cases of ischemic stroke, and 8,127 cases of CHD. In 1994 for NHS and HPFS, and 1995 for NHSII, higher egg intake was associated with higher prevalence of T2D, a higher intake of total calories, red meat, bacon, other processed meat, refined carbohydrates, potatoes, whole milk and coffee (Table 1). Higher egg intake was inversely associated with self-reported high blood cholesterol levels and statin use.

In analyses adjusted only for age and cohort, intake one or more egg per day was associated with a higher risk of CVD compared to intake of less than one egg per month (HR: 1.25, 95% CI: (1.12, 1.39)) (Table 2). After further adjustment for lifestyle and dietary characteristics associated with egg intake, the

association was attenuated and no longer statistically significant (HR: 1.10, 95% CI: (0.98, 1.23)). Similar patterns were seen in all three cohorts individually. There was no significant association between higher egg intake and risk of total CHD (p-trend=0.41) or total stroke (p-trend=0.48) when examined separately (Table 3). There was no significant interaction between egg intake and age, BMI, physical activity, smoking, high blood cholesterol or family history of MI (Table 4). We found borderline significant interactions for hypertension (p=0.05), and T2D (p=0.05). Participants who reported hypertension had a significantly increased risk of CVD with egg consumption of 1 egg or more per day (HR: 1.27, 95% CI: (1.09, 1.47)) while participants without hypertension did not (HR: 0.89, 95% CI: (0.74, 1.06)). Participants with T2D had a higher risk of CVD with consumption of one egg or more per day (HR: 1.23, 95% CI: (0.93, 1.62)) than participants without T2D (HR: 1.03, 95% CI: (0.91, 1.17)). These trends were evident in all three cohorts when examined individually. When we modeled the substitution of one egg for one serving of another food, we saw an increased risk of CVD when eggs were replaced by red and processed meat (HR: 1.09, 95% CI: (1.02, 1.17)), and a decreased risk of CVD when eggs were replaced by low-fat dairy (HR: 0.91, 95% CI: (0.86, 0.96)), whole grains (HR: 0.89, 95% CI: (0.83, 0.94)), legumes (HR: 0.91, 95% CI: (0.84, 0.99)) and nuts (HR: 0.89, 95% CI: (0.83, 0.95)) (Figure 1).

Table 1. Age-Standardized Characteristics of Participants in NHSI, NHSII and HPFS in 1994

	Whole Egg Intake								
	Nurses' Health Study I			Nurses' Health Study II			Health Professionals Follow-Up Study		
Characteristics	**0/month**	**2-4/week**	**≥1/day**	**0/month**	**2-4/week**	**≥1/day**	**0/month**	**2-4/week**	**≥1/day**
Participants, No	4,159	16,058	1,359	16,634	11,954	371	6,810	8,948	1,385
Age, mean, y	61.0	59.8	59.8	40.3	40.5	41.1	60.3	61.8	62.1
Body Mass Index*, mean, kg/m^2	25.5	27.0	27.5	24.9	26.6	27.4	25.4	26.4	26.2
Physical Activity, mean, Met h/wk	20.2	19.2	19.3	26.6	18.9	22.0	38.3	34.2	35.0
White race, %	98	98	98	96	96	94	95	95	95
Smoking Status									
Never smoker, %	40	48	43	65	67	67	54	47	42
Past smoker, %	43	40	42	25	22	20	42	43	44
Current smoker, %	17	12	15	10	11	13	4	10	14
Postmenopausal, %	90	89	89	8	7	7			
Current menopausal hormone use, %	35	37	37	6	5	4			
Current oral contraceptive use, %				9	7	8			
Family history of diabetes mellitus, %	29	30	32	16	17	19	15	14	13
Family history of myocardial infarction, %	20	18	20	13	12	10	37	29	27
High blood pressure†, %	40	40	44	9	10	13	35	34	28
High blood cholesterol†, %	50	45	45	20	16	14	48	32	28
Type 2 diabetes‡, %	4	6	8	0	1	1	4	6	9
Current multivitamin use, %	42	46	48	59	60	63	64	59	59
Current statin use, %	23	17	16	4	2	2	13	3	3
Dietary intake, mean									
Total Energy, kcal/d	1,459	1,787	1,882	1,583	2,088	2,202	1,791	2,160	2,348
Alcohol, g/d	5.8	6.4	6.5	3.1	3.5	3.1	9.3	12.3	11.5
Bacon, servings/d	0.0	0.1	0.2	0.0	0.1	0.2	0.0	0.2	0.3
Red meat, servings/d	0.9	1.0	1.1	0.5	0.9	0.9	0.5	0.9	1.1
Other processed meat, servings/d	0.2	0.2	0.3	0.1	0.2	0.3	0.1	0.3	0.3
Refined carbohydrates, servings/d	1.0	1.3	1.2	0.8	1.2	1.4	0.8	1.1	1.3
Potatoes, servings/d	0.7	0.8	0.8	0.4	0.6	0.6	0.5	0.6	0.7
Whole milk, servings/d	0.1	0.2	0.3	0.0	0.1	0.2	0.0	0.2	0.3
Coffee, servings/d	2.2	2.4	2.4	1.5	1.6	1.6	1.6	2.1	2.3
Juice, servings/d	0.5	0.5	0.5	0.6	0.8	0.8	0.8	0.8	0.8

Abbreviations: Number (No), Year (y), week (wk), servings (serv), ounces (oz), grams (g), sugar-sweetened beverages (SSB)

*Body mass index calculated as weight in kilograms divided by height in meters squared

†Includes prevalent cases before baseline and incident cases until the return of the 1994 questionnaire (1995 in NHSII)

‡Includes incident cases until the return of the 1994 questionnaire (1995 in NHSII)

Table 2. Risk of Incident Cardiovascular Disease According to Categories of Whole Egg Consumption in NHSI, NHSII and HPFS

	Frequency of Consumption Categories						p-value for trend‡	HR (95% CI) for a 1 serving per day increase
	<1/month	**1-3/month**	**1/week**	**2-4/week**	**5-6/week**	**≥1/day**		
Nurses' Health I Study								
Cases/ Person years, No.	346/106,090	845/249,856	2,748/843,273	1,446/586115	143/60,602	161/62,978		
Age adjusted model 1	1	1.10 (0.97, 1.25)	1.03 (0.92, 1.15)	1.03 (0.91, 1.15)	1.16 (0.95, 1.41)	1.25 (1.03, 1.50)	0.13	1.10 (0.97, 1.25)
Multivariable model 2*	1	1.15 (1.01, 1.30)	1.11 (0.99, 1.24)	1.09 (0.97, 1.23)	1.22 (1.00, 1.49)	1.24 (1.02, 1.49)	0.12	1.10 (0.97, 1.25)
Multivariable model 3†	1	1.13 (1.00, 1.28)	1.08 (0.96, 1.21)	1.05 (0.93, 1.19)	1.16 (0.95, 1.41)	1.19 (0.98, 1.45)	0.36	1.06 (0.93, 1.22)
Nurses' Health II Study								
Cases/ Person years, No.	167/333,857	312/659,254	394/678,556	111/274,443	10/18,036	4/10,557		
Age adjusted model 1	1	1.00 (0.83, 1.20)	1.12 (0.94, 1.35)	0.98 (0.77, 1.24)	1.23 (0.65, 2.34)	0.77 (0.29, 2.09)	0.78	1.06 (0.70, 1.60)
Multivariable model 2*	1	0.99 (0.82, 1.19)	1.10 (0.91, 1.32)	0.91 (0.72, 1.17)	1.10 (0.58, 2.10)	0.72 (0.26, 1.93)	0.70	0.92 (0.61, 1.40)
Multivariable model 3†	1	0.93 (0.77, 1.13)	1.01 (0.83, 1.23)	0.82 (0.63, 1.07)	0.97 (0.50, 1.86)	0.60 (0.22, 1.65)	0.25	0.77 (0.48, 1.21)
Health Professionals Follow-up Study								
Cases/ Person years, No.	840/139,344	1,205/203,922	2,059/308,344	1,540/238,339	212/30,784	289/38,753		
Age adjusted model 1	1	1.02 (0.94, 1.12)	1.05 (0.97, 1.14)	1.09 (1.00, 1.19)	1.19 (1.02, 1.39)	1.26 (1.10, 1.44)	<0.001	1.19 (1.09, 1.30)
Multivariable model 2*	1	1.01 (0.92, 1.10)	1.04 (0.96, 1.13)	1.05 (0.96, 1.15)	1.13 (0.97, 1.32)	1.20 (1.04, 1.37)	0.003	1.15 (1.05, 1.25)
Multivariable model 3†	1	0.98 (0.89, 1.07)	0.98 (0.90, 1.07)	0.97 (0.88, 1.07)	1.03 (0.88, 1.22)	1.09 (0.94, 1.27)	0.16	1.07 (0.97, 1.18)
Pooled Results								
Cases/ Person years, No.	1,353/579,281	2,362/1,113,032	5,201/1,830,173	3,097/1,098,897	365/109,422	454/112,288		
4Age adjusted model 1	1	1.05 (0.98, 1.12)	1.05 (0.98, 1.11)	1.06 (0.99, 1.13)	1.18 (1.05, 1.33)	1.25 (1.12, 1.39)	<0.001	1.16 (1.08, 1.25)
Multivariable model 2*	1	1.05 (0.98, 1.12)	1.06 (1.00, 1.13)	1.05 (0.98, 1.12)	1.15 (1.02, 1.29)	1.19 (1.07, 1.33)	0.003	1.12 (1.04, 1.20)
Multivariable model 3†	1	1.02 (0.95, 1.09)	1.01 (0.94, 1.07)	0.97 (0.91, 1.04)	1.05 (0.93, 1.18)	1.10 (0.98, 1.23)	0.33	1.04 (0.96, 1.13)

*Multivariable model 2 includes: age (months), sex (male, female), smoking status (never, former, current), BMI (kg/m^2: categorical <23, 23-<25, 25-<27, 27-<30, 30-<35, 35-<40, 40+), physical activity (MET-hours/week: categorical <3, 3-<9, 9-<18, 18-<27, 27+), oral contraceptive use (never, former, current), post-menopausal hormone use (premenopausal, never, former, current), race (Caucasian, other), family history of MI (yes, no), baseline high blood cholesterol (yes, no), baseline high blood pressure (yes, no) statin use (yes, no), Alcohol intake (servings/d), multivitamin use (yes, no)

†Multivariable model 3 includes: model 2 with total calories (per day: quintiles), whole milk (categorical: servings/d), bacon (categorical: servings/d), red meat (categorical: servings/d), other processed meats (categorical: servings/d), refined carbohydrates (categorical: servings/d), potatoes (categorical: servings/d), coffee (categorical: servings/d), juice (categorical: servings/d), and sugar-sweetened beverages (categorical: servings/d)

‡P-values for trend based on continuous egg variable

Table 3. Risk of Incident Cardiovascular Disease Types According to Categories of Whole Egg Consumption in NHSI, NHSII and HPFS*

Cohort		Frequency of Consumption Categories						p-value for trend†	HR (95% CI) for a 1 serving per day increase
		<1/month	1-3/month	1/week	2-4/week	5-6/week	≥1/day		
Total CHD (non-fatal MI and fatal CHD)									
NHSI	Cases/ Person years, No.	193/106,201	438/250,164	1,448/844,264	816/586,615	79/60,648	86/63,047		
	HR (95% CI)	1	1.06 (0.90, 1.26)	1.05 (0.90, 1.22)	1.04 (0.88, 1.22)	1.11 (0.84, 1.45)	1.08 (0.83, 1.41)	0.67	1.04 (0.87, 1.25)
NHSII	Cases/ Person years, No.	86/333,931	163/659,392	201/678,745	49/274,490	8/18,038	2/10,558		
	HR (95% CI)	1	0.90 (0.69, 1.18)	0.92 (0.70, 1.20)	0.63 (0.43, 0.92)	1.24 (0.58, 2.64)	0.52 (0.13, 2.17)	0.12	0.59 (0.30, 1.14)
HPFS	Cases/ Person years, No.	609/139,528	891/204,179	1,523/308,756	1,157/238,657	157/30,820	221/38,814		
	HR (95% CI)	1	0.98 (0.88, 1.09)	1.00 (0.90, 1.11)	1.00 (0.89, 1.12)	1.03 (0.85, 1.24)	1.12 (0.95, 1.33)	0.14	1.04 (0.96, 1.14)
Pooled	Cases/ Person years, No.	888/579,660	1,492/1,113,735	3,172/1,831,765	2,022/1,099,762	244/109,506	309/112,419		
	HR (95% CI)	1	1.00 (0.92, 1.09)	1.00 (0.92, 1.08)	0.98 (0.89, 1.06)	1.03 (0.88, 1.19)	1.08 (0.93, 1.24)	0.41	1.04 (0.94, 1.15)
Total Stroke									
NHSI	Cases/ Person years, No.	156/106,205	419/250,121	1,333/844,186	641/586,629	64/60,648	78/63,034		
	HR (95% CI)	1	1.23 (1.02, 1.48)	1.14 (0.96, 1.35)	1.07 (0.89, 1.28)	1.20 (0.89, 1.62)	1.36 (1.03, 1.81)	0.38	1.09 (0.90, 1.33)
NHSII	Cases/ Person years, No.	82/333,938	150/659,397	194/678,720	64/274,484	2/18,041	2/10,557		
	HR (95% CI)	1	0.96 (0.72, 1.26)	1.11 (0.84, 1.46)	1.09 (0.76, 1.56)	0.47 (0.12, 1.95)	0.69 (0.17, 2.88)	0.93	1.03 (0.55, 1.93)
HPFS	Cases/ Person years, No.	234/139,692	318/204,432	547/309,131	391/238,925	57/30,869	73/38,845		
	HR (95% CI)	1	0.96 (0.80, 1.15)	0.93 (0.79, 1.11)	0.91 (0.76, 1.10)	1.08 (0.79, 1.48)	1.08 (0.81, 1.44)	0.51	1.07 (0.89, 1.30)
Pooled	Cases/ Person years, No.	472/579,835	887/1,113,950	2,074/1,832,037	1,096/1,100,038	123/109,558	153/112,436		
	HR (95% CI)	1	1.06 (0.94, 1.18)	1.03 (0.92, 1.14)	0.97 (0.86, 1.09)	1.08 (0.88, 1.33)	1.17 (0.96, 1.42)	0.48	1.05 (0.92, 1.20)

* All results use multivariable model 3 and are adjusted for: age (months), sex (male, female), smoking status (never, former, current), BMI (kg/m^2: categorical <23, 23-<25, 25-<27, 27-<30, 30-<35, 35-<40, 40+), physical activity (MET-hours/week: categorical <3, 3-<9, 9-<18, 18-<27, 27+), oral contraceptive use (never, former, current), post-menopausal hormone use (premenopausal, never, former, current), race (Caucasian, other), family history of MI (yes, no), baseline high blood cholesterol (yes, no), baseline high blood pressure (yes, no) statin use (yes, no), Alcohol intake (servings/d), multivitamin use (yes, no), total calories (per day: quintiles), whole milk (categorical: servings/d), bacon (categorical: servings/d), red meat (categorical: servings/d), other processed meat (categorical: servings/d), refined carbohydrates (categorical: servings/d), potatoes (categorical: servings/d), coffee (categorical: servings/d), juice (categorical: servings/d), and sugar-sweetened beverages (categorical: servings/d)

† P-values for trend based on continuous egg variable derived from the median egg intake in each category of consumption

Table 4. Stratified Analysis of Risk of Incident Cardiovascular Disease According to Categories of Whole Egg Consumption in NHSI, NHSII and HPFS: Pooled Results*

Variable	Cases/Person years, No.	Frequency of Consumption Categories						p-value for trend†
		<1/month	1-3/month	1/week	2-4/week	5-6/week	≥1/day	
Age < 60	5,158/3,868,171	1	1.00 (0.90, 1.10)	0.99 (0.89, 1.09)	0.95 (0.85, 1.06)	1.01 (0.84, 1.22)	0.92 (0.77, 1.11)	0.32
Age ≥ 60	7,674/974,932	1	1.04 (0.95, 1.14)	1.02 (0.94, 1.11)	1.00 (0.91, 1.09)	1.06 (0.90, 1.25)	1.24 (1.07, 1.43)	0.03
BMI < 25	5,672/2,515,663	1	0.99 (0.89, 1.09)	1.00 (0.91, 1.09)	0.98 (0.88, 1.09)	0.95 (0.78, 1.16)	1.06 (0.89, 1.26)	0.71
BMI ≥ 25	7,160/2,327,440	1	1.05 (0.95, 1.15)	1.01 (0.93, 1.11)	0.99 (0.89, 1.09)	1.12 (0.96, 1.32)	1.12 (0.96, 1.31)	0.29
≥ 9 MET/wk PA	5,199/2,174,760	1	0.98 (0.89, 1.09)	0.98 (0.90, 1.09)	0.97 (0.87, 1.08)	1.08 (0.89, 1.31)	1.08 (0.89, 1.31)	0.45
< 9 MET/wk PA	7,633/2,668,343	1	1.04 (0.95, 1.14)	1.03 (0.94, 1.12)	0.98 (0.89, 1.08)	1.02 (0.87, 1.20)	1.13 (0.97, 1.31)	0.48
Never smoker	5,242/2,781,653	1	1.03 (0.92, 1.14)	1.07 (0.97, 1.18)	1.03 (0.93, 1.15)	1.16 (0.95, 1.41)	1.18 (0.98, 1.42)	0.08
Ever smoker	7,590/2,061,450	1	1.02 (0.93, 1.12)	0.96 (0.88, 1.05)	0.94 (0.85, 1.03)	1.00 (0.85, 1.18)	1.06 (0.92, 1.23)	0.89
No hypertension	4,780/3,403,118	1	0.99 (0.89, 1.11)	0.97 (0.87, 1.07)	0.88 (0.78, 0.98)	0.89 (0.73, 1.07)	0.88 (0.74, 1.06)	0.03
Hypertension	8,052/1,439,985	1	1.04 (0.95, 1.14)	1.04 (0.95, 1.13)	1.05 (0.96, 1.15)	1.19 (1.02, 1.41)	1.27 (1.09, 1.47)	0.001
Normal blood cholesterol	6,039/3,232,018	1	0.99 (0.89, 1.11)	1.05 (0.95, 1.16)	1.02 (0.92, 1.14)	1.07 (0.90, 1.26)	1.13 (0.97, 1.33)	0.09
High blood cholesterol	6,793/1,611,085	1	1.05 (0.96, 1.15)	0.97 (0.89, 1.06)	0.94 (0.85, 1.03)	1.08 (0.89, 1.31)	1.10 (0.92, 1.31)	0.95
No family history	8,595/3,511,746	1	1.05 (0.96, 1.14)	1.01 (0.93, 1.10)	0.98 (0.90, 1.07)	1.03 (0.88, 1.19)	1.11 (0.97, 1.28)	0.55
Family history of MI	4,237/1,331,357	1	0.96 (0.85, 1.07)	0.97 (0.87, 1.08)	0.94 (0.83, 1.06)	1.05 (0.84, 1.30)	1.06 (0.87, 1.30)	0.50
No statin use	10,636/4,412,189	1	1.05 (0.97, 1.13)	1.03 (0.96, 1.11)	0.99 (0.92, 1.07)	1.08 (0.94, 1.22)	1.11 (0.99, 1.26)	0.30
Statin use	2,196/430,914	1	0.92 (0.78, 1.07)	0.94 (0.81, 1.08)	0.94 (0.79, 1.12)	1.00 (0.67, 1.49)	1.08 (0.76, 1.52)	0.63
No T2D diagnosis	10,951/4,667,314	1	1.02 (0.95, 1.10)	1.00 (0.93, 1.07)	0.95 (0.88, 1.02)	1.02 (0.89, 1.17)	1.03 (0.91, 1.17)	0.76
T2D diagnosis	2,269/216,491	1	0.97 (0.79, 1.18)	0.96 (0.80, 1.15)	0.99 (0.82, 1.21)	1.08 (0.80, 1.46)	1.23 (0.93, 1.62)	0.05

* All results use multivariable model 3 and are adjusted for: age (months), sex (male, female), smoking status (never, former, current), BMI (kg/m^2: categorical <23, 23-<25, 25-<27, 27-<30, 30-<35, 35-<40, 40+), physical activity (MET-hours/week: categorical <3, 3-<9, 9-<18, 18-<27, 27+), oral contraceptive use (never, former, current), post-menopausal hormone use (premenopausal, never, former, current), race (Caucasian, other), family history of MI (yes, no), baseline high blood cholesterol (yes, no), baseline high blood pressure (yes, no) statin use (yes, no), Alcohol intake (servings/d), multivitamin use (yes, no), total calories (per day: quintiles), whole milk (categorical: servings/d), bacon (categorical: servings/d), red meat (categorical: servings/d), other processed meat (categorical: servings/d), refined carbohydrates (categorical: servings/d), potatoes (categorical: servings/d), coffee (categorical: servings/d), juice (categorical: servings/d), and sugar-sweetened beverages (categorical: servings/d)

† P-values for trend based on continuous egg variable derived from the median egg intake in each category of consumption

‡ P-values for interaction calculated using the likelihood ratio test

Table 4 (Continued)

Variable	Cases/Person years, No.	HR (95% CI) for a 1 serving per day increase	p-int .‡
Age < 60	5,158/3,868,171	0.94 (0.83, 1.06)	0.41
Age ≥ 60	7,674/974,932	1.12 (1.01, 1.24)	
BMI < 25	5,672/2,515,663	1.02 (0.90, 1.16)	0.51
BMI ≥ 25	7,160/2,327,440	1.06 (0.95, 1.18)	
≥ 9 MET/wk PA	5,199/2,174,760	1.04 (0.94, 1.15)	0.94
< 9 MET/wk PA	7,633/2,668,343	1.05 (0.92, 1.20)	
Never smoker	5,242/2,781,653	1.12 (0.98, 1.27)	0.63
Ever smoker	7,590/2,061,450	1.01 (0.91, 1.12)	
No hypertension	4,780/3,403,118	0.87 (0.76, 0.99)	0.05
Hypertension	8,052/1,439,985	1.18 (1.07, 1.31)	
Normal blood cholesterol	6,039/3,232,018	1.10 (0.99, 1.22)	0.18
High blood cholesterol	6,793/1,611,085	1.00 (0.88, 1.13)	
No family history	8,595/3,511,746	1.03 (0.93, 1.14)	0.88
Family history of MI	4,237/1,331,357	1.05 (0.91, 1.21)	
No statin use	10,636/4,412,189	1.05 (0.96, 1.14)	0.78
Statin use	2,196/430,914	1.06 (0.83, 1.35)	
No T2D diagnosis	10,951/4,667,314	0.99 (0.90, 1.08)	0.05
T2D diagnosis	2,269/216,491	1.21 (1.00, 1.46)	

* All results use multivariable model 3 and are adjusted for: age (months), sex (male, female), smoking status (never, former, current), BMI (kg/m^2: categorical <23, 23-<25, 25-<27, 27-<30, 30-<35, 35-<40, 40+), physical activity (MET-hours/week: categorical <3, 3-<9, 9-<18, 18-<27, 27+), oral contraceptive use (never, former, current), post-menopausal hormone use (premenopausal, never, former, current), race (Caucasian, other), family history of MI (yes, no), baseline high blood cholesterol (yes, no), baseline high blood pressure (yes, no) statin use (yes, no), Alcohol intake (servings/d), multivitamin use (yes, no), total calories (per day: quintiles), whole milk (categorical: servings/d), bacon (categorical: servings/d), red meat (categorical: servings/d), other processed meat (categorical: servings/d), refined carbohydrates (categorical: servings/d), potatoes (categorical: servings/d), coffee (categorical: servings/d), juice (categorical: servings/d), and sugar-sweetened beverages (categorical: servings/d)

† P-values for trend based on continuous egg variable derived from the median egg intake in each category of consumption

‡ P-values for interaction calculated using the likelihood ratio test

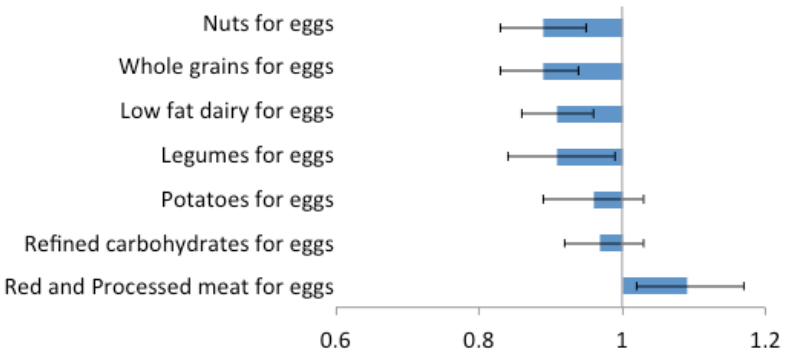


Figure 1. Hazard ratios and 95% CI's for incident CVD associated with replacement of eggs by other food groups. Adjusted for: age (months), sex (male, female), smoking status (never, former, current), BMI (kg/m^2: categorical <23, 23-<25, 25-<27, 27-<30, 30-<35, 35-<40, 40+), physical activity (MET-hours/week: categorical <3, 3-<9, 9-<18, 18-<27, 27+), oral contraceptive use (never, former, current), post-menopausal hormone use (premenopausal, never, former, current), race (Caucasian, other), family history of MI (yes, no), baseline high blood cholesterol (yes, no), baseline high blood pressure (yes, no) statin use (yes, no), Alcohol intake (servings/d), multivitamin use (yes, no), total calories (per day: quintiles)

Mean Egg intake at baseline was 0.42 eggs/day in NHS, 0.18 eggs/day in NHSII and 0.34 eggs/day in HPFS. Egg intake in NHS and HPFS decreased between 1980 and 1990, then remained constant in later years (Figure 2). In NHSII, mean egg intake was lower than in NHS and HPFS, but also remained relatively constant during follow-up.

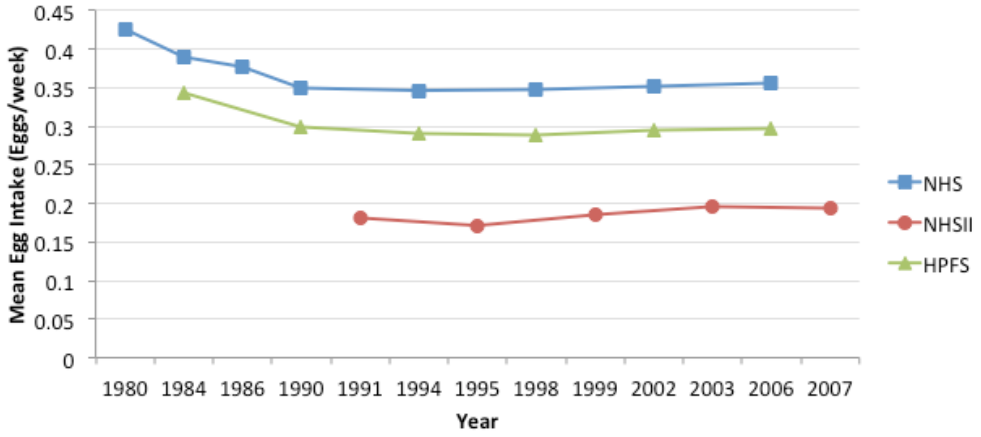


Figure 2. Trends in egg consumption over time. Mean egg intake calculated in NHS, NHSII and HPFS for each questionnaire returned.

In sensitivity analyses, we observed no significantly increased risk of CVD for participants consuming 2 or more eggs per day (HR for ≥2/day compared to <1/month: 1.04; 95% CI: 0.84, 1.30); however, there were only 94 cases of CVD for this category (Table 5). Results were similar when eggs from all sources were included (HR for ≥2/day compared to <1/month: 1.02, 95% CI: (0.85, 1.23)) (Table 6). When the risk of total stroke was examined by ischemic (2,533 cases, p-trend=0.27) and hemorrhagic stroke (750 cases, p-trend=0.22), we did not see a significantly increased risk of either (Table 7). Similarly, we did not see an increased risk with egg intake and report of CVD including CABG (21,376 cases, p-trend=0.93) (HR for ≥1/day: 1.03, 95% CI: (0.94, 1.13)). We also did not see any clinically significant changes in egg intake after diagnosis with intermediate outcomes such as T2D, hypertension, high blood cholesterol, statin use, CABG or angina, nor did we see any significant increases in risk of CVD when we stopped updating diet after diagnosis of the above intermediate outcomes (HR for ≥1/day: 1.10, 95% CI: (0.97, 1.25)). We saw no significant differences in results when used a simple update of diet each year rather than a cumulative average (HR for ≥1/day: 1.09, 95% CI: (0.98, 1.23)), or when we used baseline diet only (HR for ≥1/day: 1.07, 95% CI: (0.98, 1.18)).

Table 5. Risk of Incident Cardiovascular Disease According to Seven Categories of Egg Consumption*

	Frequency of Consumption Categories							p-value
Cohort	**<1/month**	**1-3/month**	**1/week**	**2-4/week**	**5-6/week**	**1/day**	**≥2/day**	**for trend†**
Cases/Person-years, No.	1,353/579,291	2,362/1,113,032	5,201/1,830,169	3,097/1,098,897	365/109,422	360/91,963	94/20,325	
NHS	1	1.13 (1.00, 1.28)	1.08 (0.96, 1.21)	1.05 (0.93, 1.19)	1.16 (0.95, 1.41)	1.21 (0.99, 1.47)	1.11 (0.67, 1.81)	0.50
NHSII	1	0.93 (0.77, 1.13)	1.01 (0.83, 1.23)	0.82 (0.63, 1.07)	0.97 (0.50, 1.86)	0.52 (0.16, 1.65)	1.17 (0.16, 8.54)	0.32
HPFS	1	0.98 (0.89, 1.07)	0.98 (0.90, 1.07)	0.97 (0.88, 1.07)	1.03 (0.88, 1.20)	1.11 (0.94, 1.30)	1.06 (0.83, 1.36)	0.27
Pooled Results	1	1.02 (0.95, 1.09)	1.01 (0.94, 1.07)	0.97 (0.91, 1.04)	1.05 (0.92, 1.18)	1.11 (0.98, 1.25)	1.04 (0.84, 1.30)	0.53

* All results use multivariable model 3 and are adjusted for: age (months), sex (male, female), smoking status (never, former, current), BMI (kg/m^2: categorical <23, 23-<25, 25-<27, 27-<30, 30-<35, 35-<40, 40+), physical activity (MET-hours/week: categorical <3, 3-<9, 9-<18, 18-<27, 27+), oral contraceptive use (never, former, current), post-menopausal hormone use (premenopausal, never, former, current), race (Caucasian, other), family history of MI (yes, no), baseline high blood cholesterol (yes, no), baseline high blood pressure (yes, no) statin use (yes, no), Alcohol intake (servings/d), multivitamin use (yes, no), total calories (per day: quintiles), whole milk (categorical: servings/d), bacon (categorical: servings/d), red meat (categorical: servings/d), other processed meat (categorical: servings/d), refined carbohydrates (categorical: servings/d), potatoes (categorical: servings/d), coffee (categorical: servings/d), juice (categorical: servings/d), and sugar-sweetened beverages (categorical: servings/d)

† P-values for trend based on continuous egg variable derived from the median egg intake in each category of consumption

Table 6. Risk of Incident Cardiovascular Disease According to Categories of Total Egg Consumption, Including Eggs in Mixed Foods*

	Frequency of Consumption Categories							p-value
Cohort	**<1/month**	**1-3/month**	**1/week**	**2-4/week**	**5-6/week**	**1/day**	**≥2/day**	**for trend†**
Cases/Person-years, No.	752/319,578	1,920/899,932	5,407/2,113,589	3,525/1,225,671	545/141,509	537/116,139	146/26,506	
NHS	1	1.03 (0.88, 1.21)	0.98 (0.85, 1.14)	0.97 (0.83, 1.13)	1.15 (0.94, 1.40)	1.05 (0.84, 1.30)	1.10 (0.69, 1.75)	0.45
NHSII	1	0.89 (0.69, 1.14)	0.99 (0.77, 1.28)	0.83 (0.61, 1.12)	0.65 (0.31, 1.35)	0.70 (0.28, 1.77)	1.16 (0.16, 8.47)	0.19
HPFS	1	1.01 (0.90, 1.13)	1.00 (0.90, 1.12)	1.02 (0.91, 1.15)	1.05 (0.90, 1.22)	1.06 (0.92, 1.23)	1.06 (0.86, 1.30)	0.33
Pooled Results	1	1.01 (0.92, 1.10)	1.00 (0.92, 1.08)	0.98 (0.90, 1.07)	1.06 (0.94, 1.19)	1.03 (0.92, 1.16)	1.02 (0.85, 1.23)	0.48

* All results use multivariable model 3 and are adjusted for: age (months), sex (male, female), smoking status (never, former, current), BMI (kg/m^2: categorical <23, 23-<25, 25-<27, 27-<30, 30-<35, 35-<40, 40+), physical activity (MET-hours/week: categorical <3, 3-<9, 9-<18, 18-<27, 27+), oral contraceptive use (never, former, current), post-menopausal hormone use (premenopausal, never, former, current), race (Caucasian, other), family history of MI (yes, no), baseline high blood cholesterol (yes, no), baseline high blood pressure (yes, no) statin use (yes, no), Alcohol intake (servings/d), multivitamin use (yes, no), total calories (per day: quintiles), whole milk (categorical: servings/d), bacon (categorical: servings/d), red meat (categorical: servings/d), other processed meat (categorical: servings/d), refined carbohydrates (categorical: servings/d), potatoes (categorical: servings/d), coffee (categorical: servings/d), juice (categorical: servings/d), and sugar-sweetened beverages (categorical: servings/d)

† P-values for trend based on continuous egg variable derived from the median egg intake in each category of consumption

Table 7. Risk of Incident Cardiovascular Disease Subtypes According to Categories of Egg Consumption: Pooled Results[*]

Outcome	Cases/Person years, No.	Frequency of Consumption Categories						p-value for trend[†]
		<1/month	1-3/month	1/week	2-4/week	5-6/week	≥1/day	
Total Stroke	4,805/4,847,854	1	1.06 (0.94, 1.18)	1.03 (0.92, 1.14)	0.97 (0.86, 1.09)	1.08 (0.88, 1.33)	1.17 (0.96, 1.42)	0.48
Ischemic Stroke	2,533/4,849,446	1	1.07 (0.91, 1.26)	1.00 (0.86, 1.16)	0.97 (0.82, 1.14)	1.20 (0.91, 1.59)	1.22 (0.94, 1.58)	0.27
Hemorrhagic Stroke	750/4,851,258	1	1.29 (0.95, 1.75)	1.25 (0.94, 1.67)	1.08 (0.79, 1.48)	0.96 (0.54, 1.70)	0.94 (0.54, 1.63)	0.22

[*] All results use multivariable model 3 and are adjusted for: age (months), sex (male, female), smoking status (never, former, current), BMI (kg/m^2: categorical <23, 23-<25, 25-<27, 27-<30, 30-<35, 35-<40, 40+), physical activity (MET-hours/week: categorical <3, 3-<9, 9-<18, 18-<27, 27+), oral contraceptive use (never, former, current), post-menopausal hormone use (premenopausal, never, former, current), race (Caucasian, other), family history of MI (yes, no), baseline high blood cholesterol (yes, no), baseline high blood pressure (yes, no) statin use (yes, no), Alcohol intake (servings/d), multivitamin use (yes, no), total calories (per day: quintiles), whole milk (categorical: servings/d), bacon (categorical: servings/d), red meat (categorical: servings/d), other processed meat (categorical: servings/d), refined carbohydrates (categorical: servings/d), potatoes (categorical: servings/d), coffee (categorical: servings/d), juice (categorical: servings/d), and sugar-sweetened beverages (categorical: servings/d)

[†] P-values for trend based on continuous egg variable derived from the median egg intake in each category of consumption

DISCUSSION

We observed no overall significant association between egg intake and risk of CVD after adjustment for dietary and lifestyle factors. Our observation of an increased risk of CVD when eggs were replaced with red and processed meat suggests that red and processed meat is not a healthy replacement for egg intakes. However, our results do suggest that substituting low-fat dairy, whole grains, legumes and nuts for eggs would result in a lower risk of CVD. As these foods are associated with a lower risk of CVD, consuming them in place of eggs, which do not increase or decrease risk of CVD, would result in a net lowered risk of CVD.

Our results confirm previous analyses of NHSI and HPFS published in 1999, where we did not find any significant associations between egg consumption and CVD in either men or women after adjusting for both dietary and lifestyle covariates [6]. In addition, a recent meta-analysis of prospective cohort studies found no significant association with egg intake up to one egg per day and risk of total CVD[15].

Most longitudinal cohort studies have not found any significant association between eggs and CVD. However, Djousse et al. found an increased risk of heart failure of 1.28(1.02, 1.61) for those consuming 1 egg/day and 1.64(1.08-2.49) for those consuming 2 or more eggs/day in the Physicians' Health Study after adjusting for lifestyle, but not dietary factors [29]. In a cross-sectional study of patients attending a vascular prevention clinic, Spence et al. found that egg consumption was associated with an increase in carotid plaque area; however, it is important to note that egg intake was reported retrospectively and the authors were not able to adjust for any dietary or lifestyle factors aside from smoking, leaving the potential for measurement error and residual confounding[7]. Indeed, several cohort studies that were able to adjust for lifestyle and nutrition factors found no association between egg consumption up to 1 egg per day and risk of CVD after such adjustment, similar to the current study [8, 9, 11, 13, 14, 30].

We observed a positive association between egg intake and risk of CVD among participants reporting hypertension, while there was no association in participants without hypertension. This suggests

that participants at higher risk for CVD may consider reduction of egg consumption. This relationship has not been reported in previous publications; therefore, additional studies will be needed to explore that relationship. In our cohorts, we also observed that participants with T2D had a higher risk of CVD, similar to the relationship found in previous cohort studies[6, 10]. The meta-analysis mentioned previously found a moderately increased risk of CVD among those with T2D in the highest category of egg intake compared to the lowest (HR: 1.54; 95% CI: 1.14, 2.09)[15]. It is possible that insulin resistance may contribute to altered cholesterol absorption and metabolism[31]. The altered cholesterol absorption seen in patients with T2D may increase susceptibility to CVD. In fact, we also observed that a higher percentage of participants with T2D reported hypertension and high blood cholesterol than participants without T2D.

We found no significantly elevated risk of CVD for participants consuming ≥2 eggs per day. However, the small number of cases in ≥2 eggs per day category in the current analyses limits drawing a strong conclusion from this finding. Previous publications which included a category of ≥2 eggs per day included too few cases to conclude any significant associations[9].

The current analyses have several strengths, which include repeated measurements of dietary and lifestyle variables, high rates of follow-up and a large sample size. The increase in total CVD cases from 1,124 in the previous publication of these cohorts to 12,832 in the current analyses allows for more robust findings in our main results as well as in several sensitivity analyses[6]. The repeated measurement of lifestyle variables allows for changes in risk factors over time, while the cumulative average update of dietary variables additionally limits random measurement error by accounting for within-person differences in intake. We were also able to account for many more dietary and lifestyle covariates than previous studies, including variables potentially associated with egg intake such as bacon, red and processed meat, coffee, whole milk, juice, sugar sweetened beverages, refined carbohydrates, potatoes and French fries.

This study has some limitations. First, the population consists of health professionals who are mostly of European ancestry; however, the education level of participants has likely contributed to the reliability and quality of the data collected. Second, there is some measurement error associated with

dietary data collected with the FFQ. Misclassification due to random measurement error in diet could result in an underestimation of the association between egg intake and risk of CVD; however, the cumulative average update method used for dietary variables reduces random error due to within-person variation. In addition, validation studies have found a high de-attenuated correlation for egg intake between diet measured through FFQ and 7-day weight records (0.77 for NHS and 0.80 for HPFS) [20, 21]. Third, we were not able to examine the relationship between egg whites or egg substitutes and risk of CVD. We recognize the potential for unmeasured or residual confounding, but the ability to adjust for a substantial number of dietary and lifestyle limits the likelihood for important unmeasured confounding.

In summary, in three large prospective US cohorts egg intake up to one egg per day is overall not associated with an increased risk of CVD. However, egg intake was associated with a significantly increased risk of CVD among those with hypertension or diabetes. Our analyses also suggest eggs are a healthier choice compared to red and processed meats, but that substitution of low-fat dairy, whole grains, legumes and nuts for eggs may reduce the risk of CVD.

Chapter 2

Egg consumption and risk of cardiovascular disease mortality in the AARP cohort and an updated meta-analysis of prospective cohort studies

Amanda L. Sands[1], Meir J. Stampfer[1,2,3], Frank B. Hu[1,2,3], Bernard Rosner[1,3,4], Frank M. Sacks[1,3], Rashmi Sinha[5], David Spence[6]

[1]Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, MA

[2]Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA

[3]Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA

[4]Department of Biostatistics, Harvard T.H. Chan School of Public Health, Boston, MA

[5]Nutritional Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, Department of Health and Human Services, Bethesda, MD

[6]Stroke Prevention & Atherosclerosis Research Centre, Robarts Research Institute, Western University, London, Ontario, Canada

ABSTRACT

Background

Due to their high dietary cholesterol content, limiting eggs has been recommended for prevention of cardiovascular disease (CVD).

Objective

To evaluate the association between egg intake up to 2 eggs per day and the risk of Cardiovascular Disease (CVD) mortality in the NIH-AARP Diet and Health study, a large prospective cohort. In addition, to perform a meta-analysis of prospective cohort studies of egg intake and risk of CVD.

Methods

After exclusions, the final population for analysis included 134,731 men and 111,015 women without CVD, cancer or type 2 diabetes (T2D) at baseline. Dietary and lifestyle factors including egg intake were assessed through two questionnaires at baseline. Cardiovascular disease mortality was determined from ICD codes found through linkage to the Social Security Administration Death Master File and National Death Index. Deaths through December 13st, 2011 were included in the analysis. Multivariate cox proportional hazards models were used to estimate relative risks (HR) and 95% confidence intervals (CI). An updated meta-analysis of the current literature was also conducted.

Results

We identified 11,268 cases of CVD mortality. We observed the following risk of CVD mortality with egg intake (HR(95%CI) by category: 0-1/month: 1(Ref); 2-3/month: 0.94 (0.89, 1.00); 1-2/week: 0.99 (0.93, 1.04); 3-4/week: 1.04 (0.97, 1.10); 5-6/week: 1.08 (0.97, 1.19); 1/day: 1.23 (1.06, 1.42); ≥2/day: 1.14 (0.95, 1.36); p-trend<0.001). For each increase of one egg per day we found that the risk of CVD mortality was (HR: 1.12, 95% CI: (1.05, 1.20)). Among participants with T2D, there was an increased risk of CVD mortality for each increase of one egg per day, with HR: 1.25 (1.11, 1.41). Our updated meta-analysis, which includes 630,786 participants and 28,139 cases of CVD, found a small increased risk of CVD with increases in egg intake of 1 egg per day in the fixed-effect model (HR: 1.05;

95% CI(1.01, 1.09), p-value for heterogeneity=0.052). Among participants with T2D a fixed effects meta-analysis found an increased risk of CVD for an increase in consumption of 1 egg per day with HR: 1.24 (1.12, 1.37) (p-value for heterogeneity=0.78).

Conclusions

Higher egg intake was associated with a modestly increased risk of CVD after adjustment for lifestyle factors in the AARP Study and meta-analysis. There was a significant positive association between egg intake and risk of CVD among participants with T2D.

INTRODUCTION

Cardiovascular disease is the leading cause of death worldwide.[24] Dietary and lifestyle factors play a large part in the incidence and progression of the disease.[1] In the past, decreases in egg consumption have been recommended due to the cholesterol contained in eggs (about 200g).[1] However, dietary cholesterol does not have as large of an effect on blood cholesterol levels as other dietary factors such as saturated and unsaturated fats.[44, 45] The 2013 AHA/ACC report concluded that there was "insufficient evidence to determine whether lowering dietary cholesterol reduces LDL-C[46]. In addition, the 2015 Dietary Guidelines Advisory Committee did not carry forward the recommendations of an upper limit for dietary cholesterol.[3] In addition, eggs contain a number of other beneficial nutrients such as B-vitamins and unsaturated fat[4].

Recent articles have linked egg intake to risk factors for cardiovascular disease. A cross-sectional study of vascular prevention clinic patients found an increase in atherosclerotic plaque area with increasing egg consumption.[7] In addition, recent research on gut microbiota has found a link between higher levels of TMAO, a metabolite of lecithin which is found in egg yolks, and CVD in at risk patients.[47]

Although these studies and others have sparked debate, several prospective studies of egg consumption have found no material increased risk of CVD. A recent meta-analysis found that an increase in consumption of one egg per day did not increase the risk of coronary heart disease (CHD) (HR: 0.99; 95% CI:((0.85, 1.15)) or stroke (HR: 0.91; 95% CI:(0.81, 1.02)).[27] Additionally, an updated analysis of the Nurses' Health and Health Professionals Follow-up Studies did not find a significantly increased risk of incident CVD with egg consumption of one or more eggs per day (HR: 1.10; 95% CI:(0.98, 1.23)).[48] Several other studies found no increased risk of either stroke or CHD mortality with increasing egg intake.[10, 12, 13]

The aim of the current analysis is to determine the relationship between egg intake and risk of CVD in an older population at risk for CVD. The large sample size allows us to examine egg intake of 2

or more eggs per day, whereas most previous publications have only been able to determine risk for 1 egg per day or less.

METHODS

The NIH-AARP Diet and Health Study is a prospective cohort study that began in 1995 and included members of the AARP living in six U.S. states (California, Florida, Pennsylvania, New Jersey, North Carolina or Louisiana) and two metropolitan areas (Atlanta, Georgia and Detroit, Michigan). Baseline questionnaires collected self-report information on diet and lifestyle factors on 567,169 participants. Diet was collected through validated semi-quantitative FFQ. The FFQ was compared to 24-hour recalls from a subset of 1,415 patients and correlations between the individual foods in the FFQ and 24-hour recalls ranged from 0.42 for fiber to 0.70 for red meat.[49] The correlation between the FFQ and 24-hour recalls for dietary cholesterol was 0.68 for both men and women[50]. An additional survey was completed by 334,906 of the baseline participants and included risk factor information such as medication use, physical activity and family history of disease in 1996.

Assessment of Egg Intake

Egg intake was reported with the baseline validated semi-quantitative FFQ in 1995. Participants were asked on average how many eggs, of any type, they consumed in the past 12 months in categories ranging from never to two or more times per day. Participants were additionally asked what type of egg they normally consumed (whole, egg whites or egg substitutes) and the usual cooking method. Total number of eggs consumed was evenly divided between each of the egg types reported (whole, egg whites or egg substitutes) to calculate the final whole egg intake variable. Reported intake includes whole eggs prepared separately from other foods. Egg intake was analyzed in categories of 0-1 per month, 2-3 per month, 1-2 per week, 3-4 per week, 5-6 per week, 1 per day and ≥2 per day.

Assessment of CVD Mortality

Mortality was determined through linkage to the Social Security Administration Death Master File (SSA DMF) and through searches of the National Death Index (NDI). Cause of death was determined through death certificate ICD codes that were further grouped into National Cancer Institute Surveillance Epidemiology and End Results (SEER) codes. Cardiovascular disease mortality includes the following categories: diseases of heart, hypertension without heart disease, cerebrovascular diseases, atherosclerosis, aortic aneurysm and dissection, and other diseases of arteries, arterioles and capillaries.

Statistical Methods

Participants were excluded if they used a proxy to complete questionnaires (n=19,139), reported prevalent cancer at baseline (n=4,551), did not complete the risk factor questionnaire or left the study before returning the risk factor questionnaire (n= 222,660), reported diabetes (n=27,468), heart disease (n=36,386) or stroke (n=3,286) at baseline, or if BMI was missing (n=5,059). Unrealistic total energy intake was determined by calculating the interquartile range from the first to third quartile of the Box-Cox normally transformed total energy variable[51]. Any values less than two times the interquartile range below the first quartile or above the third quartile were excluded (n=1,999). The final sample for analysis included 245,746 participants (134,731 men and 111,015 women). Covariates that were considered as possible confounders for use in multivariate analysis include: age, race/ethnicity, education, BMI, smoking status, physical activity, diabetes history, family history of CVD, self-reported health, hypertension, alcohol consumption, multivitamin use, total energy intake and for women; postmenopausal status, use of menopausal hormones, and use of oral contraceptives. In addition, an overall healthy diet (aHEI), western dietary pattern, glycemic load, and foods associated with egg intake (e.g. bacon, red meat, other processed meats, refined carbohydrates, potatoes, whole milk, juice, sugar-sweetened beverages and coffee) were considered as possible confounders. For missing covariates, a missing indicator category was created and included in the model. Total energy intake was included in the final model to control for confounding and reduce extraneous variation due to body size, physical activity and

metabolic efficiency. Hazard ratios and 95% confidence intervals were calculated using multivariate adjusted Cox proportional Hazards analyses for incident CVD mortality. Person time was calculated from the date of return of the risk factor questionnaire in 1996 until death or the end of follow-up on December 31, 2011. Stratified analyses and likelihood ratio tests for interactions were conducted for age, sex, race, BMI, physical activity, smoking status, baseline hypertension, baseline high blood cholesterol and egg cooking method.

In sensitivity analyses, eggs including egg whites or substitutes and total eggs (including eggs from mixed dishes) were analyzed separately as exposure variables. To examine the possibility of recall bias due to changes in disease status, we conducted secondary analyses for the population including those with CVD and T2D at baseline. The proportional hazards assumption was confirmed through a likelihood ratio test of a model including of a time to event interaction term and a model without the interaction term (p-value=0.07).

Additionally, the recent meta-analysis by Rong et al. was updated to include the current results.[27] PubMed and Embase were searched through June 2015 using the following key words: "Cardiovascular diseases" MeSH, CVD, coronary heart disease, CHD, "Coronary disease" MeSH, "myocardial infarction" MeSH, MI, "Stroke" MeSH and egg. Relative risks for one egg per day were calculated for each study using the trend for log relative risks of egg intake categories, accounting for correlated estimates.[38, 52] Dose of egg consumption was the median of each category or midpoint of the upper and lower bound of each egg category. If HRs 95% CIs and person-years were not available, summary statistics were used based on the highest category of egg consumption compared to the lowest. We examined heterogeneity using the Cochran Q test and I^2 statistic. We used fixed effects when heterogeneity between studies was low and random effects when heterogeneity was high. All p-values are 2-sided. Statistical analyses for the meta-analysis were performed using Stata v.12 (StataCorp, College Station, Tx). All other statistical analyses were performed using SAS v.9.3 (SAS Institute, Inc., Cary, NC).

RESULTS

After all exclusions, there were 245,746 participants and 11,268 cases of CVD mortality in the final sample. Higher egg intake at baseline was associated with higher BMI, number of participants reporting high blood cholesterol, total energy, and intake of bacon, red meat, other processed meats, coffee and sugar-sweetened beverages in both men and women. Higher egg intake was also associated with lower education level, less married participants, more smokers and lower levels of physical activity in men and women. Additionally, higher egg consumption was associated with increased percentage of participants with a family history of T2D, lower multivitamin use, higher intake of refined carbohydrate and whole milk in men, and a lower percentage of participants with a family history of T2D in women (Table 8).

In analyses adjusted only for age, higher egg consumption was associated with a significantly increased risk of CVD mortality (1/day HR: 1.84, 95% CI(1.59, 2.13); ≥2/day HR: 2.14, 95% CI(1.80, 2.54); p-trend<0.001) (Table 9). However, after adjustment for diet and lifestyle factors, results were substantially attenuated (1/day HR: 1.23, 95% CI(1.06, 1.42); ≥2/day HR: 1.14, 95% CI(0.95, 1.36); p-trend<0.001). After multivariate adjustment, every increase of one egg per day was associated with an increased risk of CVD mortality of HR: 1.12, 95% CI(1.05, 1.20). A significant interaction was found for race (p=0.01), with a relatively greater increased risk of CVD mortality as egg consumption increased for non-whites compared to whites (Table 10). No other significant interactions were found for age, sex, BMI, physical activity, smoking status, hypertension, high blood cholesterol, or egg cooking method.

Table 8: Baseline Age-Standardized Characteristics According to Whole Egg Consumption Levels

	Whole Egg Intake					
	Women			Men		
	0-1/month	3-4/week	≥2/day	0-1/month	3-4/week	≥2/day
Participants, No	36,881	11,529	361	34,004	22,408	1,380
Age, mean, y	62.6	62.3	61.8	62.7	62.7	62.6
Body mass Index, mean*	25.7	27.4	28.5	26.1	27.6	28.2
White race, %	91	92	93	93	94	91
Some college education, %	60	58	52	76	66	56
Married, %	46	44	43	83	84	77
Smoking Status						
Never smoker, %	45	41	29	37	27	21
Past smoker, %	41	37	42	54	56	53
Current smoker, %	10	18	24	5	14	23
Current Physical Activity >1/month, %	68	62	24	77	71	65
Current menopausal hormone use, %	47	46	39			
Postmenopausal, %	95	95	94			
Oral contraceptive use >1yr, %	39	42	39			
Self-reported Good Health Status, %	91	89	88	94	92	87
Multivitamin use ≥1/wk, %	56	54	60	50	45	45
Hypertension, %	32	35	33	35	36	34
High Blood Cholesterol, %	42	56	68	37	52	61
Family History of Diabetes, %	28	28	31	23	25	27
Dietary Intake, mean						
Total energy, kcal/day	1420	1825	2027	1825	2289	2702
Alcohol, serv/day	0.4	0.6	0.4	1.1	1.6	1.6
Bacon, oz/day	0.0	0.0	0.1	0.0	0.1	0.2
Red meat, oz/d	1.0	1.9	2.5	1.7	3.1	3.9
Other processed meat, oz/day	0.4	0.5	0.7	0.6	1.0	1.2
Refined carbohydrates products, oz/day	3.4	4.1	3.8	4.4	4.9	5.3
Potatoes, oz/day	0.3	0.4	0.4	0.4	0.5	0.6
Whole milk, cups/day	0.0	0.1	0.2	0.1	0.2	0.4
Coffee, cups/day	2.8	3.4	3.6	3.0	3.9	4.6
Juice, cups/day	0.7	0.7	0.7	0.9	0.8	0.8
Sugar-sweetened beverages, oz/day	0.2	0.3	0.5	0.3	0.6	0.8

Abbreviations: Number (No), Year (y), week (wk), servings (serv), ounces (oz), grams (g)

*Body mass index calculated as weight in kilograms divided by height in meters squared

Table 9: Cardiovascular Disease Mortality According to Categories of Whole Egg Consumption

Variable	Frequency of Consumption Categories							p-trend	HR (95%CI) for increase of 1 egg/day
	0-1/month	2-3/month	1-2/week	3-4/week	5-6/week	1/day	≥2/day		
Cases, No.	2,967	2,250	3,331	1,866	526	193	135		11,268
Person years, No.	953,762	736,142	980,815	445,855	105,250	34,284	21,977		3,278,085
Age adjusted model 1	1	1.02 (0.96, 1.07)	1.11 (1.06, 1.17)	1.38 (1.31, 1.47)	1.71 (1.56, 1.87)	1.84 (1.59, 2.13)	2.14 (1.80, 2.54)	<0.001	1.70 (1.61, 1.80)
Multivariable model 2*	1	0.96 (0.90, 1.01)	1.01 (0.96, 1.06)	1.09 (1.02, 1.15)	1.16 (1.05, 1.27)	1.33 (1.15, 1.54)	1.28 (1.07, 1.52)	<0.001	1.21 (1.14, 1.29)
Multivariable model 3†	1	0.94 (0.89, 1.00)	0.99 (0.93, 1.04)	1.04 (0.97, 1.10)	1.08 (0.97, 1.19)	1.23 (1.06, 1.42)	1.14 (0.95, 1.36)	<0.001	1.13 (1.05, 1.20)

*Multivariable model 2 includes: age (years), sex, race (Caucasian, non-Hispanic black, other), education (high school, some college, college, unknown), marriage status (married, widowed, divorced, separated, never married, unknown), BMI (kg/m^2: categorical <23, 23-<25, 25-<27, 27-<30, 30-<35, 35-<40, ≥40), smoking dose (never, past, current: 1-19/d, current: ≥20/d, unknown), physical activity (≥20 minutes: categorical never, rarely, 1-3/mo, 1-2/wk, 3-4/wk, ≥5/wk, unknown), menopausal status (pre, post-menopausal unknown), post-menopausal hormone use (never, former, current, unknown), oral contraceptive use (years: categorical 0, 1-4, 5-9, ≥10, unknown), self-reported health status (excellent, very good, good, fair, poor, unknown), baseline hypertension (yes, no), baseline high blood cholesterol (yes, no), alcohol intake (servings/day: quintiles), multivitamin use (never, <1/wk, 1-3/wk, 4-6/wk, daily, unknown)

†Multivariable model 3 includes: model 2 with total calories (per day), bacon (servings/d: quintiles), red meat (servings/d: quintiles), other processed meat (servings/d: quintiles), refined carbohydrates (servings/d: quintiles), potatoes (servings/d: quintiles), whole milk (servings/d: quartiles), coffee (servings/d: quintiles), juice (servings/d: quintiles), sugar-sweetened beverages (servings/d: quintiles)

Table 10. Stratified Analysis of Cardiovascular Disease Mortality According to Categories of Whole Egg Consumption*

Variable	Frequency of Consumption Categories							p-trend†
	0-1/month	2-3/month	1-2/week	3-4/week	5-6/week	1/day	≥2/day	
Age < 70	1	0.93 (0.87, 0.98)	0.98 (0.92, 1.04)	1.03 (0.96, 1.10)	1.08 (0.97, 1.40)	1.25 (1.07, 1.46)	1.17 (0.97, 1.40)	<0.001
Age ≥ 70	1	1.04 (0.90, 1.20)	1.02 (0.89, 1.18)	1.05 (0.88, 1.25)	1.05 (0.78, 1.41)	1.08 (0.70, 1.66)	0.91 (0.51, 1.63)	0.84
Male	1	0.91 (0.85, 0.98)	0.97 (0.91, 1.04)	1.03 (0.96, 1.12)	1.07 (0.95, 1.20)	1.20 (1.00, 1.43)	1.07 (0.88, 1.31)	0.007
Female	1	0.98 (0.90, 1.07)	1.00 (0.92, 1.09)	1.02 (0.91, 1.15)	1.07 (0.86, 1.31)	1.32 (1.00, 1.75)	1.48 (0.98, 2.25)	0.03
White	1	0.92 (0.87, 0.98)	0.97 (0.92, 1.03)	1.02 (0.95, 1.09)	1.03 (0.92, 1.14)	1.16 (0.99, 1.36)	1.12 (0.93, 1.35)	0.009
Other	1	1.18 (0.95, 1.45)	1.13 (0.92, 1.39)	1.24 (0.98, 1.57)	1.74 (1.26, 2.41)	1.91 (1.24, 2.94)	1.35 (0.74, 2.47)	0.004
BMI < 30	1	0.93 (0.87, 0.99)	0.99 (0.93, 1.06)	1.05 (0.98, 1.13)	1.03 (0.91, 1.17)	1.20 (1.00, 1.43)	1.15 (0.92, 1.43)	0.009
BMI ≥ 30	1	0.98 (0.87, 1.10)	0.96 (0.86, 1.07)	1.01 (0.89, 1.14)	1.20 (1.01, 1.42)	1.35 (1.02, 1.78)	1.19 (0.88, 1.60)	0.01
< 20 min/wk PA	1	1.01 (0.92, 1.11)	1.02 (0.93, 1.11)	1.14 (1.02, 1.26)	1.19 (1.02, 1.38)	1.33 (1.06, 1.68)	1.16 (0.88, 1.53)	0.002
≥ 20 min/wk PA	1	0.91 (0.85, 0.97)	0.97 (0.91, 1.04)	0.98 (0.90, 1.06)	1.01 (0.89, 1.15)	1.16 (0.96, 1.41)	1.13 (0.89, 1.42)	0.07
Non-smoker	1	0.94 (0.89, 1.00)	0.98 (0.93, 1.04)	1.02 (0.95, 1.09)	1.10 (0.98, 1.23)	1.22 (1.03, 1.45)	1.18 (0.96, 1.45)	0.002
Current smoker	1	0.92 (0.81, 1.06)	1.00 (0.88, 1.14)	1.09 (0.94, 1.26)	1.03 (0.84, 1.26)	1.25 (0.92, 1.69)	1.08 (0.77, 1.52)	0.10
No hypertension	1	0.93 (0.86, 1.00)	0.97 (0.90, 1.05)	1.02 (0.94, 1.12)	1.04 (0.91, 1.19)	1.21 (0.99, 1.49)	1.03 (0.80, 1.31)	0.09
Hypertension	1	0.96 (0.89, 1.04)	1.00 (0.92, 1.08)	1.05 (0.96, 1.15)	1.11 (0.97, 1.28)	1.24 (0.99, 1.54)	1.28 (0.99, 1.65)	0.002
Normal blood cholesterol	1	0.96 (0.89, 1.04)	1.01 (0.95, 1.09)	1.06 (0.97, 1.15)	1.03 (0.89, 1.19)	1.16 (0.92, 1.46)	1.11 (0.85, 1.47)	0.07
High blood cholesterol	1	0.93 (0.85, 1.01)	0.96 (0.88, 1.05)	1.02 (0.93, 1.13)	1.14 (0.99, 1.31)	1.30 (1.07, 1.58)	1.18 (0.93, 1.49)	<0.001
Cooked without fat	1	0.98 (0.91, 1.05)	0.99 (0.92, 1.06)	1.04 (0.95, 1.13)	1.11 (0.95, 1.29)	1.14 (0.91, 1.43)	1.02 (0.75, 1.37)	0.16
Cooked with added fat	1	0.93 (0.85, 1.02)	1.00 (0.92, 1.10)	1.06 (0.96, 1.17)	1.08 (0.94, 1.24)	1.32 (1.08, 1.62)	1.24 (0.99, 1.55)	<0.001

* All results are from multivariable model 3 and include: age (years), sex, race (Caucasian, non-Hispanic black, other), education (high school, some college, college, unknown), marriage status (married, widowed, divorced, separated, never married, unknown), BMI (kg/m^2: categorical <23, 23-<25, 25-<27, 27-<30, 30-<35, 35-<40, ≥40), smoking dose (never, past, current: 1-19/d, current: ≥20/d, unknown), physical activity (≥20 minutes: categorical never, rarely, 1-3/mo, 1-2/wk, 3-4/wk, ≥5/wk, unknown), menopausal status (pre, post-menopausal unknown), post-menopausal hormone use (never, former, current, unknown), oral contraceptive use (years: categorical 0, 1-4, 5-9, ≥10, unknown), self-reported health status (excellent, very good, good, fair, poor, unknown), hypertension (yes, no), high blood cholesterol (yes, no), alcohol intake (servings/day: quintiles), multivitamin use (never, <1/wk, 1-3/wk, 4-6/wk, daily, unknown), total calories (per day), bacon (servings/d: quintiles), red meat (servings/d: quintiles), other processed meat (servings/d: quintiles), refined carbohydrates (servings/d: quintiles), potatoes (servings/d: quintiles), whole milk (servings/d: quartiles), coffee (servings/d: quintiles), juice (servings/d: quintiles), sugar-sweetened beverages (servings/d: quintiles)

Table 10 (Continued)

Variable	Cases	HR (95% CI) for increase of 1 egg/day	p-int[‡]
Age < 70	9,640/3,072,301	1.14 (1.06, 1.22)	0.40
Age ≥ 70	1,628/205,784	1.02 (0.84, 1.24)	
Male	7,256/1,776,763	1.11 (1.03, 1.20)	0.50
Female	4,012/1,501,322	1.17 (1.02, 1.34)	
White	10,489/3,049,639	1.10 (1.02, 1.18)	0.01
Other	779/228,446	1.38 (1.11, 1,71)	
BMI < 30	8,458/2,669,482	1.15 (1.04, 1.29)	0.58
BMI ≥ 30	2,810/608,603	1.24 (1.05, 1.47)	
< 20 min/wk PA	4,099/972,807	1.18 (1.06, 1.30)	0.31
≥ 20 min/wk PA	7,169/2,305,278	1.09 (0.99, 1.19)	
Non-smoker	8,170/2,012,144	1.13 (1.05, 1.22)	0.62
Current smoker	3,098/1,265,941	1.12 (0.98, 1.28)	
No hypertension	5,734/2,187,406	1.08 (0.99, 1.19)	0.67
Hypertension	5,534/1,090,679	1.17 (1.06, 1.29)	
Normal blood cholesterol	6,296/1,714,305	1.10 (0.99, 1.21)	0.51
High blood cholesterol	4,972/1,563,780	1.17 (1.07, 1.28)	
Cooked without fat	5,876/1,712,711	1.08 (0.97, 1.19)	0.76
Cooked with added fat	5,401/1,565,374	1.18 (1.08, 1.30)	

[†]P-values for trend based on continuous egg variable derived from the median egg intake in each category of consumption
[‡]P-values for interaction calculated using the likelihood ratio test

In sensitivity analyses we observed a relatively higher increased risk of CVD mortality in those consuming two or more eggs per day who reported having T2D at baseline (HR: 1.53, 95% CI: (1.15, 2.03)) compared to those who had no T2D or CVD at baseline (HR: 1.14, 95% CI: (0.95, 1.36), p-value for interaction=0.63) (Table 11). We also saw an increased risk of CVD mortality for two or more eggs per day in those who reported both baseline CVD and T2D (HR: 1.60, 95% CI(1.12, 2.29)). However, for those reporting CVD without T2D at baseline, the corresponding HR (95% CI) was 0.66 (0.44, 1.00). We observed no significant differences in risk of CVD mortality when we limited follow-up time to 5 or to 10 years to explore the possibility of misclassification due to baseline collection of dietary intakes (data not shown). Compared to whole eggs, the inclusion of eggs from all sources was associated with a similar risk of CVD mortality (HR for an increase of 1 egg/day: 1.11, 95% CI(1.04, 1.19))(Table 12), as was the inclusion of egg whites and egg substitutes with whole eggs as the exposure (HR for an increase of 1 egg/day: 1.13, 95% CI(1.06, 1.21)) (Table 13).

Table 11. Cardiovascular Disease Mortality According to Categories of Whole Egg Consumption Including Participants who Reported Heart Disease, Stroke or Type 2 Diabetes at Baseline*

	No baseline T2D or CVD	Baseline T2D Only	Baseline CVD Only	Baseline T2D and CVD
Cases/Person years, No.	11,268/3,278,085	2,174/223,269	6,186/470,900	2,323/85,307
Frequency of Consumption Categories				
0-1/month	1	1	1	1
2-3/month	0.94 (0.89, 1.00)	1.07 (0.93, 1.22)	0.99 (0.91, 1.06)	0.95 (0.84, 1.08)
1-2/week	0.99 (0.93, 1.04)	1.04 (0.92, 1.19)	1.00 (0.93, 1.07)	1.00 (0.93, 1.07)
3-4/week	1.00 (0.93, 1.07)	1.21 (1.05, 1.39)	1.06 (0.97, 1.16)	1.05 (0.92, 1.20)
5-6/week	1.08 (0.97, 1.19)	1.18 (0.97, 1.45)	1.05 (0.90, 1.23)	1.13 (0.92, 1.39)
1/day	1.23 (1.06, 1.42)	1.28 (0.97, 1.69)	0.98 (0.74, 1.28)	1.13 (0.82, 1.57)
≥2/day	1.14 (0.95, 1.36)	1.53 (1.15, 2.03)	0.66 (0.44, 1.00)	1.60 (1.12, 2.29)
p-trend†	<0.001	<0.001	0.73	0.004
HR (95% CI) for increase of 1 egg/day	1.13 (1.05, 1.20)	1.25 (1.11, 1.41)	0.98 (0.88, 1.10)	1.23 (1.07, 1.42)

* All results are from multivariable model 3 and include: age (years), sex, race (Caucasian, non-Hispanic black, other), education (high school, some college, college, unknown), marriage status (married, widowed, divorced, separated, never married, unknown), BMI (kg/m^2: categorical <23, 23-<25, 25-<27, 27-<30, 30-<35, 35-<40, ≥40), smoking dose (never, past, current: 1-19/d, current: ≥20/d, unknown), physical activity (≥20 minutes: categorical never, rarely, 1-3/mo, 1-2/wk, 3-4/wk, ≥5/wk, unknown), menopausal status (pre, post-menopausal unknown), post-menopausal hormone use (never, former, current, unknown), oral contraceptive use (years: categorical 0, 1-4, 5-9, ≥10, unknown), self-reported health status (excellent, very good, good, fair, poor, unknown), hypertension (yes, no), high blood cholesterol (yes, no), alcohol intake (servings/day: quintiles), multivitamin use (never, <1/wk, 1-3/wk, 4-6/wk, daily, unknown), total calories (per day), bacon (servings/d: quintiles), red meat (servings/d: quintiles), other processed meat (servings/d: quintiles), refined carbohydrates (servings/d: quintiles), potatoes (servings/d: quintiles), whole milk (servings/d: quartiles), coffee (servings/d: quintiles), juice (servings/d: quintiles), sugar-sweetened beverages (servings/d: quintiles)

†P-values for trend based on continuous egg variable derived from the median egg intake in each category of consumption

Table 12. Cardiovascular Disease Mortality According to Categories of Egg Consumption from Whole Eggs and Eggs in Mixed Dishes

	Frequency of Consumption Categories								
Variable	**0-1/month**	**2-3/month**	**1-2/week**	**3-4/week**	**5-6/week**	**1/day**	**≥2/day**	**p-trend†**	**HR (95% CI) for increase of 1 egg/day**
Cases, No.	357	1,267	5,663	2,203	1,105	537	136		11,268
Person years, No.	101,360	417,784	1,802,436	595,236	239,933	99,280	22,056		3,278,085
Multivariable model 3*	1	0.90 (0.80, 1.01)	0.90 (0.80, 1.01)	0.91 (0.80, 1.03)	1.00 (0.88, 1.15)	1.05 (0.90, 1.22)	1.04 (0.85, 1.28)	0.003	1.11 (1.04, 1.18)

*Results are from multivariable model 3 and include: age (years), sex, race (Caucasian, non-Hispanic black, other), education (high school, some college, college, unknown), marriage status (married, widowed, divorced, separated, never married, unknown), BMI (kg/m^2: categorical <23, 23-<25, 25-<27, 27-<30, 30-<35, 35-<40, ≥40), smoking dose (never, past, current: 1-19/d, current: ≥20/d, unknown), physical activity (≥20 minutes: categorical never, rarely, 1-3/mo, 1-2/wk, 3-4/wk, ≥5/wk, unknown), menopausal status (pre, post-menopausal unknown), post-menopausal hormone use (never, former, current, unknown), oral contraceptive use (years: categorical 0, 1-4, 5-9, ≥10, unknown), self-reported health status (excellent, very good, good, fair, poor, unknown), hypertension (yes, no), high blood cholesterol (yes, no), alcohol intake (servings/day: quintiles), multivitamin use (never, <1/wk, 1-3/wk, 4-6/wk, daily, unknown), total calories (per day), bacon (servings/d: quintiles), red meat (servings/d: quintiles), other processed meat (servings/d: quintiles), refined carbohydrates (servings/d: quintiles), potatoes (servings/d: quintiles), whole milk (servings/d: quartiles), coffee (servings/d: quintiles), juice (servings/d: quintiles), sugar-sweetened beverages (servings/d: quintiles)

†P-values for trend based on continuous egg variable derived from the median egg intake in each category of consumption

Table 13. Cardiovascular Disease Mortality According to Categories of Egg Consumption Including Whole Eggs, Egg Whites and Egg Substitutes

Variable	Frequency of Consumption Categories							p-trend[†]	HR (95% CI) for increase of 1 egg/day
	0-1/month	2-3/month	1-2/week	3-4/week	5-6/week	1/day	≥2/day		
Cases, No.	2,060	2,536	3,674	2,037	582	219	160		11,268
Person years, No.	658,549	845,207	1,094,632	493,873	118,549	40,217	27,059		3,278,085
Multivariable model 3[a]	1	0.94 (0.88, 0.99)	0.98 (0.93, 1.04)	1.02 (0.96, 1.10)	1.06 (0.97, 1.17)	1.21 (1.05, 1.39)	1.14 (0.97, 1.35)	0.003	1.13 (1.06, 1.20)

[a] Results are from multivariable model 3 and include: age (years), sex, race (Caucasian, non-Hispanic black, other), education (high school, some college, college, unknown), marriage status (married, widowed, divorced, separated, never married, unknown), BMI (kg/m^2: categorical <23, 23-<25, 25-<27, 27-<30, 30-<35, 35-<40, ≥40), smoking dose (never, past, current: 1-19/d, current: ≥20/d, unknown), physical activity (≥20 minutes: categorical never, rarely, 1-3/mo, 1-2/wk, 3-4/wk, ≥5/wk, unknown), menopausal status (pre, post-menopausal unknown), post-menopausal hormone use (never, former, current, unknown), oral contraceptive use (years: categorical 0, 1-4, 5-9, ≥10, unknown), self-reported health status (excellent, very good, good, fair, poor, unknown), hypertension (yes, no), high blood cholesterol (yes, no), alcohol intake (servings/day: quintiles), multivitamin use (never, <1/wk, 1-3/wk, 4-6/wk, daily, unknown), total calories (per day), bacon (servings/d: quintiles), red meat (servings/d: quintiles), other processed meat (servings/d: quintiles), refined carbohydrates (servings/d: quintiles), potatoes (servings/d: quintiles), whole milk (servings/d: quartiles), coffee (servings/d: quintiles), juice (servings/d: quintiles), sugar-sweetened beverages (servings/d: quintiles)

[†] P-values for trend based on continuous egg variable derived from the median egg intake in each category of consumption

In the updated meta-analysis we retrieved 1,660 articles from the preliminary search. After review of the articles, 1,630 were eliminated if they did not mention CVD or eggs, and 24 were excluded after full text review due to being reviews or commentaries, not including CVD as the outcome, not including sufficient data for meta-analysis or if they were previous publications of studies that were already included in the meta analysis (Figure 3).

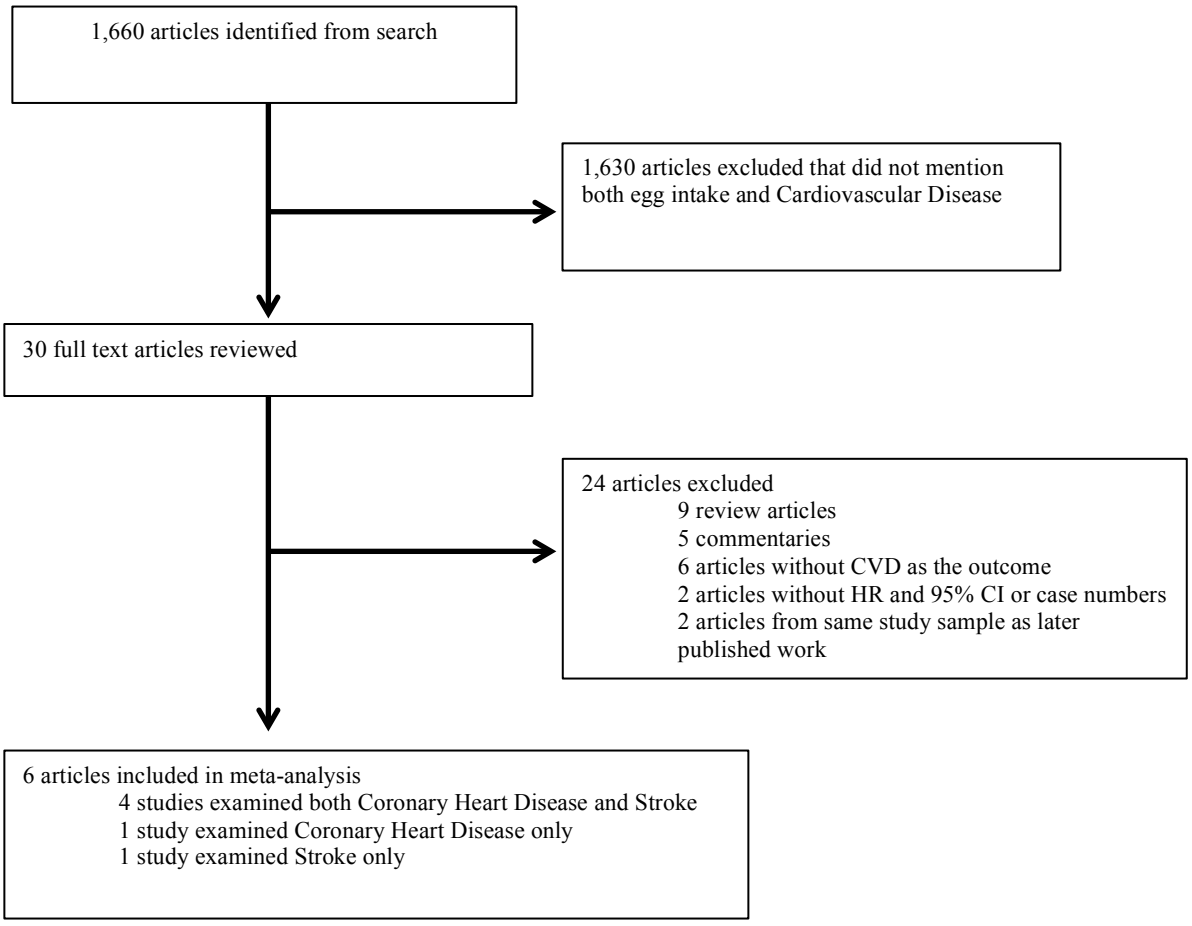


Figure 3. Meta-analysis Search Strategy and Study Selection

Table 14. Meta-analysis Study Characteristics

Study	Population	Country	sex	Age at baseline	# participants	mean follow-up	Case ascertainment
Sauvaget, 2003	Hiroshima/Nagasaki Life Span Study	Japan	Male and Female	34-103	34807	16-17	Confirmed by nationwide family registration system
Nakamura, 2004	NIPPON DATA80	Japan	Male	30+	4077	14	Confirmed by National Vital Statistics
			Female	30+	5186	14	Confirmed by National Vital Statistics
Nakamura, 2006	Japan Public Health Center-based prospective study	Japan	Male and Female	40-69	90735	10.2	Confirmed by medical records, letter, telephone or death certificate
Quershi, 2007	NHANES-I	USA	Male and Female	25-74	9734	20	Confirmed by medical records or death certificate

Table 14 (Continued)

Study	Population	Endpoints	Relative risks	Covariates in fully adjusted model
Sauvaget, 2003	Hiroshima/Nagasaki Life Span Study	stroke mortality (1259)	Never, 1.0 (reference); ≤1 egg/week*, 0.74 (0.55 to 1.00); 2-4 eggs/week, 0.78 (0.59 to 1.04); almost daily, 0.72 (0.54 to 0.97)	Age, sex, birth cohort, city, radiation dose, body mass index, smoking, alcohol habits, education level, history of diabetes, or hypertension
Nakamura, 2004	NIPPON DATA80	Ischemic heart disease mortality (39)	Ischemic heart disease mortality: seldom, 1.18 (0.26 to 5.42); 1-2 eggs/week, 1.71 (0.78 to 3.76); 0.5 eggs/day, 1.49 (0.63 to 3.48); 1 egg/day, 1.0 (reference)	Age, serum creatinine, total cholesterol, blood glucose, body mass index, blood pressures, use of blood pressure lowering drugs, smoking, and alcohol intake
		stroke mortality (112)	Stroke mortality: seldom, 0.93 (0.36 to 2.40); 1-2 eggs/week, 1.09 (0.69 to 1.72); 0.5 eggs/day, 1.10 (0.68 to 1.76); 1 egg/day, 1.0 (reference); ≥2 eggs/day, 0.25 (0.03 to 1.81)	
		Ischemic heart disease mortality (41)	Ischemic heart disease mortality: seldom, 1.42 (0.56 to 3.62); 1-2 eggs/week, 0.64 (0.28 to 1.44); 0.5 eggs/day, 0.78 (0.35 to 1.82); 1 egg/day, 1.0 (reference);	Age, serum creatinine, total cholesterol, blood glucose, body mass index, blood pressures, use of blood pressure lowering drugs, smoking, and alcohol intake
		stroke mortality (107)	Stroke mortality: seldom, 0.78 (0.35 to 1.73); 1-2 eggs/week, 0.79 (0.47 to 1.33); 0.5 eggs/day, 1.46 (0.89 to 2.4); 1 egg/day, 1.0 (reference); ≥2 eggs/day, 1.22 (0.29 to 5.17) ≥2 eggs/day, 1.27 (0.16 to 9.80)	
Nakamura, 2006	Japan Public Health Center-based prospective study	Coronary heart disease (462)	<1 egg/week*, 1.19 (0.86 to 1.64); 1-2 eggs/week, 1.00 (0.77 to 1.30); 3-4 eggs/week, 1.00 (0.79 to 1.26); almost daily, 1.0 (reference)	Age; sex; body mass index; hypertension; diabetes; use of cholesterol lowering drugs; smoking; alcohol drinking; whether participants intended to avoid cholesterol rich diets; consumption frequencies of meat, fish, vegetables, and fruits; and cohort effects
Quershi, 2007	NHANES-I	Coronary artery disease (1584)	Coronary artery disease: <1 egg/week, 1.0 (reference); 1-6, 1.0 (0.9 to 1.1); >6, 1.1 (0.9 to 1.3)	Age, sex, race or ethnicity, systolic blood pressure, diabetes mellitus, serum cholesterol, smoking, body mass index, and educational status
		stroke (655)	Stroke: <1 egg/week, 1.0 (reference); 1-6, 0.9 (0.7 to 1.0); >6, 0.9 (0.7 to 1.1)	

Table 14 (Continued)

Study	Population	Country	sex	Age at baseline	# participants	mean follow-up	Case ascertainment
Djousse, 2008	Physician's Health Study I	USA	Male	40-85	21327	20	Confirmed by physicians or medical records
Scrafford, 2010	NHANES III	USA	Male	17+	6833	8.8	Not applicable
			Female	17+	8113	8.9	Not applicable
Sands, 2015	Health Professionals Follow-up Study	USA	Male	40-75	42,961	20.4	Self-reported diagnosis or confirmed by medical records or autopsy

Table 14 (Continued)

Study	Population	Endpoints	Relative risks	Covariates in fully adjusted model
Djousse, 2008	Physician's Health Study I	Myocardial infarction (1550)	Myocardial infarction: <1 egg/week, 1.0 (reference); 1, 1.12 (0.96 to 1.31); 2-4, 1.16 (1.00 to 1.36); 5-6, 1.18 (0.93 to 1.49); ≥7, 0.90 (0.72 to 1.14)	Age, body mass index, smoking, history of hypertension, vitamin intake, alcohol consumption, vegetable consumption, breakfast cereal, physical activity, treatment arm, atrial fibrillation, diabetes mellitus, hypercholesterolemia, and parental history of premature myocardial infarction
		stroke (1342)	Stroke:<1egg/week,1.0(reference);1,0.96(0.82to 1.13);2-4,1.06(0.91to1.24);5-6,1.13(0.89to1.42); ≥7, 0.99 (0.80 to 1.23)	
Scrafford, 2010	NHANES III	Coronary heart disease mortality (198)	Coronary heart disease mortality: <1 egg/week, 1.0 (reference); 1-6, 1.26 (0.79 to 2.00); >6, 1.13 (0.61 to 2.11)	Age, energy, marital status, educational status, race or ethnicity, smoking, body mass index, waist to hip ratio, diabetes, hypertension, and dietary variables
		stroke mortality (63)	Stroke mortality: <1 egg/week, 1.0 (reference); 1-6, 1.00 (0.49 to 2.02); >6, 0.27 (0.10 to 0.73)	
		Coronary heart disease mortality (168)	Coronary heart disease mortality: <1 egg/week, 1.0 (reference); 1-6, 1.12 (0.66 to 1.89); >6, 0.92 (0.27 to 3.11)	Age, energy, marital status, educational status, race or ethnicity, smoking, body mass index, waist to hip ratio, diabetes, hypertension, and dietary variables
		stroke mortality (74)	Stroke mortality: <1 egg/week, 1.0 (reference); 1-6, 0.93 (0.46 to 1.90); >6, 1.03 (0.25 to 4.22)	
Sands, 2015	Health Professionals Follow-up Study	Cardiovascular disease (4,270)	<1 egg/month, 1.0 (reference); 1-3/month,0.93(0.85, 1.01); 1/week, 0.91(0.84, 0.99); 2-4/week, 0.93(0.85, 1.03); 5-6/week, 0.86(0.71, 1.03); ≥1/day, 1.07(0.91, 1.25)	age, smoking, BMI, physical activity, race, family history of CVD, high blood cholesterol at baseline, high blood pressure at baseline, statin use at baseline, alcohol intake, multivitamin use, calories, whole milk, bacon, other red and processed meat, refined grains, coffee

Table 14 (Continued)

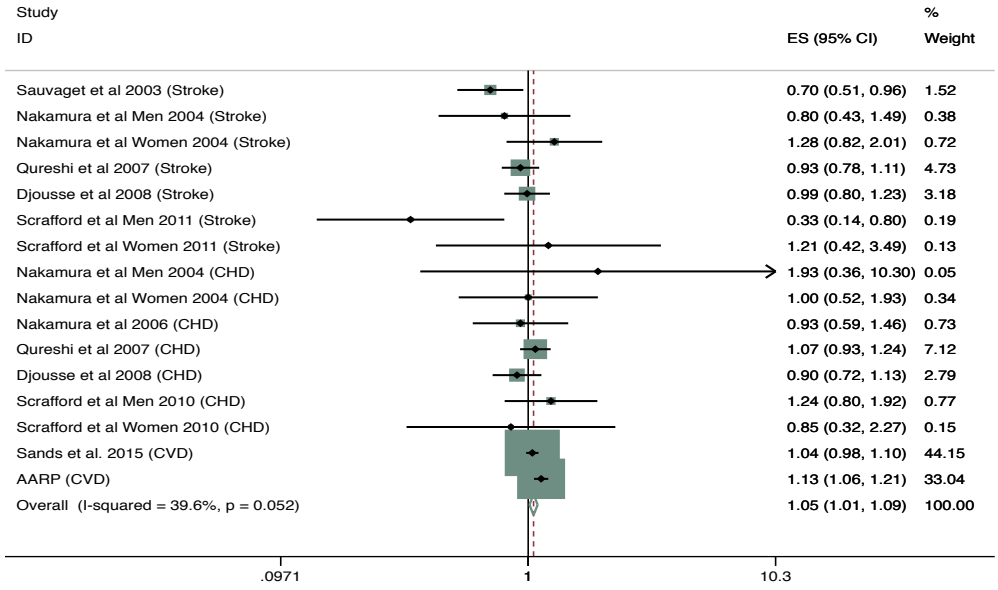
Study	Population	Country	sex	Age at baseline	# participants	mean follow-up	Case ascertainment
	Nurses' Health Study	USA	Female	34-59	70,235	27.3	Self-reported diagnosis or confirmed by medical records or autopsy
	Nurses' Health Study II	USA	Female	25-42	91,032	19.6	Self-reported diagnosis or confirmed by medical records or autopsy

Table 14 (Continued)

Study	Population	Endpoints	Relative risks	Covariates in fully adjusted model
	Nurses' Health Study	Cardiovascular disease (3,188)	<1 egg/month, 1.0 (reference); 1-3/month, 1.03(0.91, 1.17); 1/week, 0.96(0.86, 1.07); 2-4/week, 0.91(0.81, 1.03); 5-6/week, 1.04(0.86, 1.27); ≥1/day, 1.15(0.95, 1.40)	age, smoking, BMI, physical activity, race, family history of CVD, high blood cholesterol at baseline, high blood pressure at baseline, statin use at baseline, postmenopausal hormone use, alcohol intake, multivitamin use, calories, whole milk, bacon, other red and processed meat, refined grains, coffee
	Nurses' Health Study II	Cardiovascular disease (521)	<1 egg/month, 1.0 (reference); 1-3/month, 0.92(0.76, 1.11); 1/week,1.02(0.85, 1.22); 2-4/week,0.87(0.67, 1.14); 5-6/week, 0.70(0.32, 1.52); ≥1/day, 0.83(0.33, 2.07)	age, smoking, BMI, physical activity, race, family history of CVD, high blood cholesterol at baseline, high blood pressure at baseline, statin use at baseline, postmenopausal hormone use, oral contraceptive use, alcohol intake, multivitamin use, calories, whole milk, bacon, other red and processed meat, refined grains, coffee

In the final analysis, four studies were included that examined both stroke and coronary heart disease risk, one study was included that examined only coronary heart disease risk and one study was included that examined only stroke risk (Table 14). We found a modestly increased risk of CVD with an increase in egg intake of 1 egg per day (HR: 1.05, 95% CI(1.01, 1.09)) using fixed effects meta-analysis, and no increased risk (HR: 1.01, 95% CI(0.94, 1.09)) using random effects (Figure 4). We found no association with eggs in the studies that examined incident CVD (HR: 1.02, 95% CI(0.98, 1.07)), but did find an increased risk with an increase of one egg per day in studies reporting on cardiovascular disease mortality (HR: 1.10, 95% CI(1.03, 1.17)) (Figure 5). Among participants with type 2 diabetes we saw a significantly increased risk of CVD for an increase in one egg per day (HR: 1.24; 95% CI(1.12, 1.37)); However, this is based on only two studies due to missing information on case numbers and person-time in other publications.

A.



B.

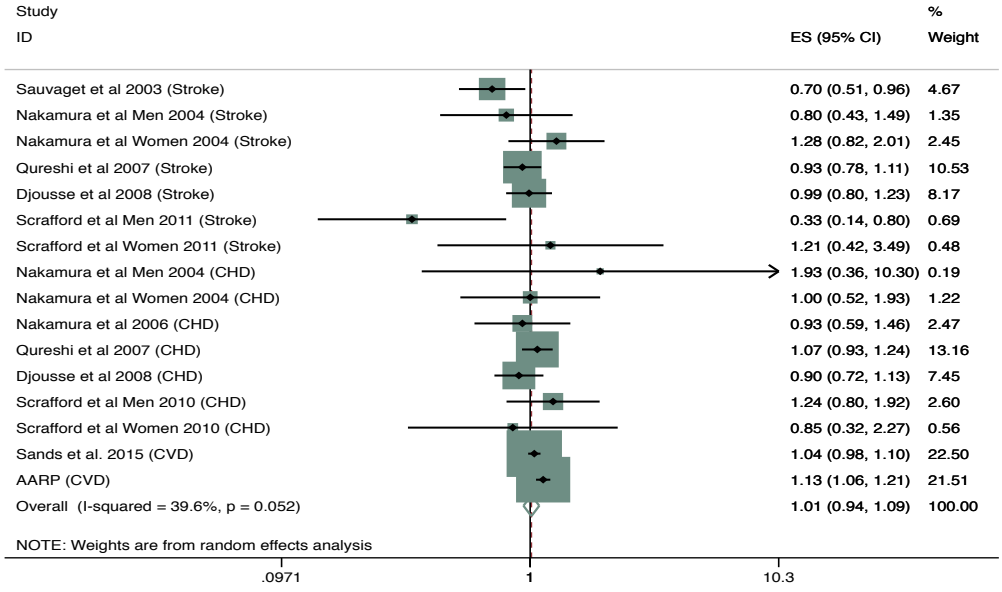
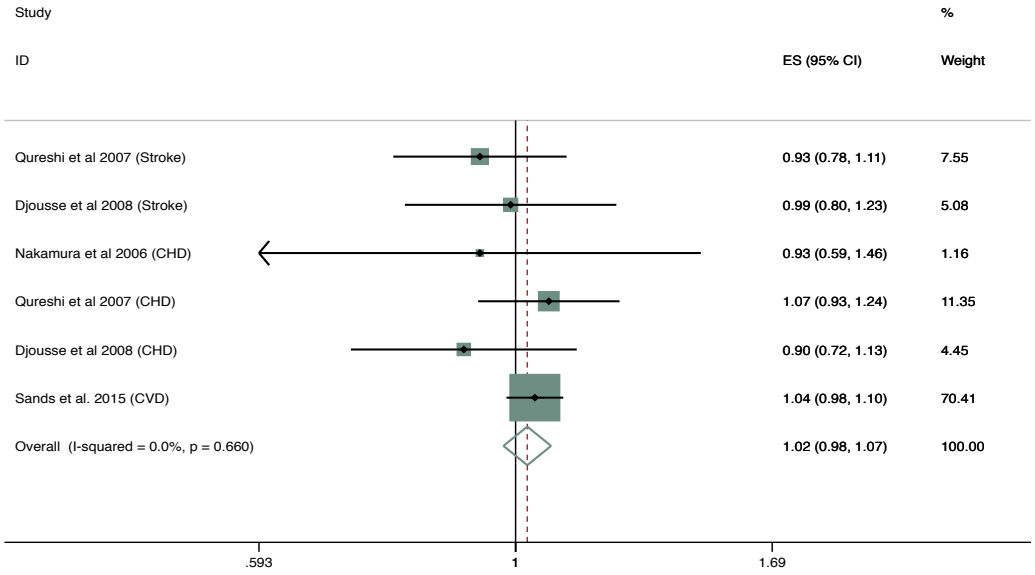


Figure 4. Risk of Cardiovascular Disease with Consumption of 1 egg/day using fixed (A) and random (B) effects meta-analysis. Weights of each of the studies are represented by the size of the square. Black diamonds represent the individual study effects and black lines represent the 95% confidence intervals. The overall effect estimate and 95% confidence interval is represented by the diamond and dotted line.

A.



B.

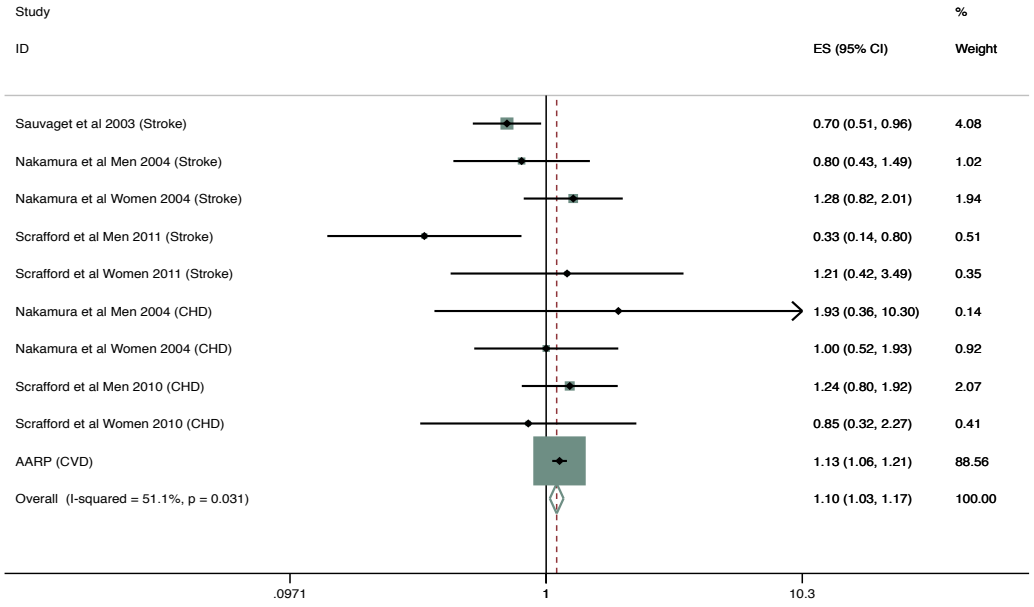


Figure 5. Risk of Incident Cardiovascular Disease (A) and Cardiovascular Disease Mortality (B) with Consumption of 1 egg/day. Weights of each of the studies are represented by the size of the square. Black diamonds represent the individual study effects and black lines represent the 95% confidence intervals. The overall effect estimate and 95% confidence interval is represented by the diamond and dotted line.

DISCUSSION

In this sample of 245,746 men and women in the NIH-AARP Diet and Health study, we observed a slight increased risk of CVD mortality associated with consumption of one egg or more per day intake after adjustment for confounders. Among participants with T2D at baseline, an increase in egg intake was significantly associated with an increased risk of CVD mortality.

In the updated meta-analysis, there was a similarly modest increase in risk of CVD with increases in egg consumption of 1 egg per day using fixed effects meta-analysis but not with random-effects. Random-effects meta-analysis allows the true risk to vary between studies and tends to give more weight to smaller studies than fixed-effects meta-analysis. Although there is a moderate amount of heterogeneity between the studies included, it is not statistically significant (p=0.05); therefore, the fixed-effects may be more representative of the true association. In the meta-analysis, we also observed a significantly increased risk of CVD with egg consumption among those with T2D.

Although we found a modestly significant association between an increase in consumption of 1 egg per day and risk of CVD in the AARP cohort, it is possible that some of this association may be the result of unmeasured confounding in participants at higher risk due to T2D. We saw an increased risk of CVD among participants reporting T2D at baseline; misclassification due to more undiagnosed or unreported T2D in the one egg per day group may be driving the association. Our findings of an increased risk of CVD among participants with T2D are consistent with the previously published meta-analysis which found an increased risk of CHD (HR: 1.54; 95% CI:1.14, 2.09) in the highest category of egg intake compared to the lowest among participants with T2D.[27] It has been hypothesized that the increased levels of insulin resistance and changes in alipoprotein levels seen with T2D (specifically decreased apolipoprotein E and increased apolipoprotein CII) may lead to changes in lipoprotein transport and HDL metabolism that result in an increased risk of CVD.[53-56]

To date, no other studies have reported a significant interaction with race. In the current study, there were 93.1% non-Hispanic white, 3.0% non-Hispanic black, 1.6% Hispanic, 1.1% Asian, 0.1% pacific Islander and 0.2% American Indian participants. Those with the highest risk were non-Hispanic blacks, who had an increased risk of CVD mortality with an increase of one egg per day of (HR: 1.63, 95% CI: (1.22, 2.20)). More research is needed to confirm or refute this finding.

In the past, limiting dietary cholesterol was recommended for the reduction of blood cholesterol levels; however, this hypothesized relationship has not been confirmed in the literature. Indeed, both the AHA/ACC 2013 guidelines and the 2015 Dietary Guidelines Committee Report do not include a recommended upper limit for dietary cholesterol due to the lack of a clear relationship between dietary and serum cholesterol[3, 46]. In 2007-2008, the average dietary cholesterol intake in US adults measured through NHANES was 298 mg/d, which corresponds to about 1.5 eggs if no other sources of dietary cholesterol are consumed[57]. Although eggs contain a large amount of dietary cholesterol, variation in their intake appears to have little effect on serum total or LDL cholesterol levels in people consuming moderate amounts of dietary cholesterol[26]. Intake of saturated fat is a much stronger driver of serum LDL[58]. In addition to dietary cholesterol, eggs contain a variety of nutrients beneficial to health such as polyunsaturated fats and B-vitamins[4].

The AARP cohort has several strengths. The large sample size and diverse population allows for robust results and the ability to examine risk status stratified by race, T2D, and other factors. In addition, we were able to better control for confounding by dietary factors commonly consumed with eggs, such as bacon, red and processed meat, potatoes, refined carbohydrates, coffee, whole milk, sugar sweetened beverages and juice, than in previous analyses. In this and many western populations, those who consume more eggs tend to have a host of other unfavorable risk factors; thus, inadequate control for confounding would tend to lead to exaggerated risk associated with egg intake.

The current analyses also have several limitations. First, the population includes only those older than 50; therefore, results may not be generalizable to younger populations. However, this may also be viewed as a strength because the population is at higher risk for CVD, allowing for greater power to detect an association than previous publications due to a higher number of cases. Second, because CVD incidence was not collected during follow-up, we were only able to examine the risk of CVD mortality. Third, there is some degree of misclassification due to random measurement error in the measurement of diet as a result of using the FFQ. The manner in which egg intake was assessed on the FFQ, which asks for usual type of egg intake, could also introduce additional random measurement error. The lack of dietary collection after baseline may also increase the possibility of misclassification if there are changes in diet after baseline that are not captured. This misclassification could lead to a decreased association between egg intake and the risk of CVD. In addition, because diagnosis of T2D was only reported at baseline, our analyses may include some unreported cases of T2D. We cannot eliminate the possibility of residual or unmeasured confounding in our analyses despite having adjusted for many important covariates.

Higher egg intake was associated with a modestly increased risk of CVD mortality after adjustment for diet and lifestyle factors in the AARP Study. A similar association was seen for the risk of CVD in the meta-analysis of current prospective cohort studies. There was a significant positive association between egg intake and risk of CVD among participants with T2D and in non-white participants. Taken together, these results suggest that eggs are associated with little increased overall risk of cardiovascular mortality, but specific population subgroups – such as those with T2D may consider limiting egg intake.

Chapter 3

Egg consumption and risk of type 2 diabetes: findings from three large prospective US cohort studies of men and women and a meta-analysis of prospective cohort studies

Amanda L. Sands, Yanping Li[1], Frank M. Sacks[1,2], Bernard Rosner[1,2,3], JoAnn E. Manson[2,4], Walter C. Willett[1,2,5], Meir J. Stampfer[1,2,5], Frank B. Hu[1,2,5]

[1]Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, MA

[2]Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA

[3]Department of Biostatistics, Harvard T.H. Chan School of Public Health, Boston, MA

[4]Preventive Medicine, Department of Medicine, Brigham and Women's Hospital, Boston, MA

[5]Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA

ABSTRACT

Background

In the current literature, there is no clear consensus on whether egg consumption is associated with the risk of type 2 diabetes (T2D).

Objective

The goal of this study is to determine the association between egg intake and risk of T2D in three large prospective cohorts, and to perform a meta-analysis of prospective cohort studies.

Design

The final study population includes 42,479 men from the Health Professionals Follow-up Study (HPFS: 1986-2010), 69,480 women from the Nurses' Health Study (NHS: 1980-2010) and 90,905 women from the Nurses' Health Study II (NHSII: 1991-2011). Participants were excluded if they reported cancer, cardiovascular disease (CVD) or T2D at baseline. Egg intake was assessed via a validated food frequency questionnaire every four years. Incident T2D was self-reported and confirmed through a validated supplemental questionnaire. We used Cox proportional hazard models to estimate relative risks (HR) and 95% confidence intervals (CI). Final multivariate models were adjusted for age, lifestyle and dietary factors. We also meta-analyzed these cohorts with previously published prospective cohorts.

Results

There were 16,570 incident cases of T2D in NHS, NHSII and HPFS. After multivariate adjustment, hazard ratios (95% confidence interval) by categories of egg intakes were: (HR(95% CI) by category: <1/month: 1(reference); 1-3/month: 1.02(0.96, 1.08); 1/week: 1.02(0.96, 1.08); 2-4/week: 1.08(1.01, 1.15); 5-6/week: 1.18(1.06, 1.32); 1+/day: 1.03(0.92, 1.16) (p-trend=0.03). Using a continuous variable for egg intake, an increase of one egg per day was associated with an increased risk of T2D of (HR: 1.09, 95% CI: (1.01, 1.18)). In substitution analysis models, replacing eggs with legumes, low-fat dairy, nuts and whole grains was associated with lower risk of T2D by 9-18%, while replacing eggs with red and processed meats was associated with increased risk by 9%. In the meta-analysis of 9 prospective cohort studies (348,965 participants and 23,823 incident cases), we found a significantly increased risk of

T2D with an increase in egg consumption of 1 egg/day (HR: 1.13, 95% CI: (1.07, 1.19)) using fixed effects. However, this increased risk was no longer present when random effects were used (HR: 1.04, 95% CI: (0.88, 1.23) (p-heterogeneity<0.001).

Conclusions

After controlling for lifestyle and dietary factors, higher egg intake (up to one egg per day) was associated with a small increased risk of incident T2D our cohorts. We found a similar increase in risk of T2D in an updated meta-analysis using fixed effects, but not when using random effects. Replacing eggs with legumes, low-fat dairy, nuts and whole grains was associated with a decreased risk of T2D; however, replacing eggs with red and processed meat was associated with an increased risk of T2D.

INTRODUCTION

In the US, it is estimated that there are over 25.6 million adults living with Type 2 Diabetes (T2D) [24]. Lifestyle factors including overweight and obesity, physical activity, smoking status, alcohol consumption and diet play an important role in the development of T2D [1, 2]. Consumption of polyunsaturated fatty acids may reduce risk of T2D, while saturated and trans fats may increase risk [5].

Several studies have focused on the relationship between egg consumption, or dietary cholesterol and risk of T2D, but the results have been inconsistent. Djousse et al. found that in the Women's Health and Physician's Health cohort studies, egg intakes of ≥7 times per week was associated with increased risk of T2D [59], but this relationship was not significant in other cohort studies [16, 17, 19-21, 60]. Two meta-analyses reported a significant positive association between egg intake and T2D risk (14) [61]. However, one of the meta-analyses also included case-control studies (potentially prone to recall bias) and two recently published studies have not been included in either meta-analysis [19, 21].

The current study examines the association between egg consumption and risk of T2D in three large ongoing cohort studies: The Nurses' Health Study (NHS), the Nurses' Health Study II (NHS II), and the Health Professionals' Follow-up Study (HPFS). Of the previously published studies, few have been able to adjust sufficiently for confounding by dietary variables. We also conducted an updated meta-analysis of our results from these three cohorts as well as six previously published prospective cohort studies.

METHODS

NHS began in 1976 with 121,701 female registered nurses between the ages of 30-55. NHSII began in 1989 with 116,430 female registered nurses between the ages of 25-44. HPFS began in 1986 with 51,529 male health professionals between the ages of 40-75. Self-administered validated questionnaires with information on disease diagnoses, health and lifestyle were completed every two years. Diet was measured through validated semi-quantitative food frequency questionnaires (FFQ) every 4 years beginning in 1980 for NHSI, 1991 for NHSII and 1986 for HPFS [28-30]. Follow-up rates were over

90%. Exclusion criteria included diagnosis with cancer, cardiovascular disease or T2D prior to baseline. Participants were also excluded if they were missing egg intake or total calories at baseline and if they reported unrealistic intake on the FFQ (>70 items blank, <500 or >3500 kcals/day for women and <800 or >4200 kcals/day for men). After exclusions, 69,383 participants in NHS, 90,903 participants in NHSII and 42,465 participants in HPFS were included in the analyses. The Institutional Review Board of the Brigham and Women's Hospital in Boston approved all study protocols.

Assessment of Egg Intake

Dietary information was collected through a semi-quantitative FFQ with 61 items in 1980 and 131 items beginning in 1984. The FFQ was administered every 4 years beginning in 1990 for NHS, 1991 for NHSII and 1986 for HPFS. Each cycle, participants were asked how often on average they consumed eggs in the past year. Responses could range from never to six or more times per day. FFQ's were validated against 7 day weighed food records in 127 men [33] and 173 women [32]. Pearson correlation coefficients corrected for within-person variation for the FFQ and 7 day weighed record ranged from 0.17 for spinach to 0.94 for yogurt and beer [32]. The de-attenuated correlation for egg intake was 0.77 in women and 0.80 in men [32, 33]. For the primary analysis, egg intake was divided into the following six categories: 0-1 per month, 2-3 per month, 1 per week, 2-4 per week, 5-6 per week and ≥1 per day. In secondary analysis an additional category of ≥2 per day was added.

Assessment of T2D

Self-report of T2D diagnosis was collected via questionnaires every two years. Diagnoses were confirmed using a supplemental questionnaire, which asked about symptoms, medication use and diagnostic tests. This supplemental questionnaire was validated with 62 cases in NHS and 59 cases in HPFS. Of these self-reported T2D diagnoses, endocrinologists blinded to risk factor status confirmed T2D from review of medical records in 98% of NHS cases and 97% of HPFS cases [62, 63]. Diabetes was defined as one or more classic symptoms (excessive thirst, polyuria, weight loss, hunger, pruritus, or

coma) plus the NDDG cut-points of fasting plasma glucose ≥140mg/dL or random plasma glucose ≥200mg/dL until 1997 and the ADA cut-points of fasting plasma glucose ≥126mg/dL or random plasma glucose ≥200mg/dL after 1997; or at least two elevated plasma glucose levels on two occasions in the absence of symptoms; or treatment with hypoglycemic medication [64]. We included confirmed cases only. After report of death, the date of death was confirmed from medical records, the National Death Index, tumor registries and death certificates.

Statistical Methods

Participants returned the first dietary questionnaire in 1980 in NHS, 1991 in NHSII and 1986 in HPFS. Follow up began with the return of the first questionnaire and ended with diagnosis with T2D, death, lost to follow-up, or the return of the last questionnaire prior to June 30th, 2010 in NHS, June 30th 2011 in NHSII and January 31st 2010 in HPFS. Cox proportional hazards models were used to calculate hazard ratios and 95% confidence intervals for incident T2D. We confirmed the assumption of proportional hazards with a likelihood ratio test based on inclusion of a time to event interaction term. To represent long-term diet and reduce within-person variation a cumulative average update method was used for egg intake and other dietary variables [37]. Whole eggs did not include eggs prepared within mixed dishes such as baked goods. A trend across categories of egg consumption was calculated from the median of each category of egg intake [38].

Multivariate models included adjustment for age, cohort, two year time intervals, sex, race/ethnicity, BMI, smoking status, physical activity, family history of T2D, diagnosis with hypertension or hypercholesterolemia at baseline, alcohol consumption, multivitamin use, postmenopausal status, use of postmenopausal hormones, use of oral contraceptives, total energy intake, and foods associated with egg intake including: bacon, red meat, other processed meat, whole milk, refined carbohydrates, potatoes, coffee, juice and sugar sweetened beverages. Western dietary pattern score was also considered as a covariate but ultimately not included in the final model due to overlap with the individual foods. As with egg intake, a cumulative average update was used for all dietary variables. Interactions were tested using

likelihood ratio tests, and stratified analyses were performed for updated age, BMI, physical activity, hypertension, high blood cholesterol, smoking and family history of T2D.

In sensitivity analyses, the joint effect of eggs and BMI on incident T2D was calculated using three categories of BMI (<25, 25-29.9, ≥30) and four categories of egg intake (<3/month, 1/week, 2-4/week, ≥5/week). P-values for interactions of the joint effect of eggs and BMI were determined from likelihood ratio tests. We determined the risk of incident T2D in seven categories of egg intake (including 2 or more eggs per day) and in overall egg intake (including eggs from mixed foods). To determine the best model to minimize measurement error, and also account for the latency period of disease, we also repeated analyses with simple update of diet and baseline diet rather than cumulative average intake. In addition, the change in egg intake after diagnosis with hypertension, high blood cholesterol, angina, CABG or beginning statin use was examined, and analyses were conducted where we stopped updating diet after diagnosis with hypertension, high blood cholesterol or beginning statin use to explore the possibility of confounding due to diagnosis with an intermediate endpoint. We also examined the effects of substituting eggs by 1 serving of other common food groups on risk of T2D. We included continuous variables for both eggs and the other food in the model along with other non-dietary confounders. We used the differences between the beta-coefficients, variance and the covariance of eggs and the other food to calculate HR's and 95% CI's for incident T2D. All p-values were two-sided and statistical analyses were performed using SAS v.9.3 (SAS Institute, Inc., Cary, NC).

We conducted a meta-analysis based on the current study and previous literature. We used the key words: "Diabetes Mellitus"[MeSH], "Diabetes Mellitus, Type 2"[MeSH], diabetes, T2D, insulin resistance and egg to search PubMed and Embase through June, 2015. We calculated Relative risks for one egg per day using the trend for log relative risks, which accounted for correlated estimates[38, 52]. To determine dose of egg consumption we used the median of each egg intake category if available, or the midpoint between the upper and lower bound. For studies that did not publish person-years for each category of egg intake, we imputed person years based on available data. Fixed effects were used when heterogeneity was low, otherwise random effects were used. Heterogeneity was assessed using the

Cochran Q test and I^2 statistic. Statistical analysis for the meta-analysis was performed using Stata v.12 (StataCorp, College Station, TX).

RESULTS

During follow-up, we recorded 7,728 cases of T2D after 28 years of follow-up in NHS, 5,345 cases after 20 years of follow-up in NHSII and 3,497 cases after 22 years of follow-up in HPFS. We found that in 1994 for NHS and HPFS and 1995 for NHSII, higher egg intake was associated with less reported high blood cholesterol and statin use, and higher intakes of total calories, red meat, processed meat, bacon, potatoes, whole milk, coffee and dietary cholesterol. (Table 15).

In analyses adjusted for age alone, higher egg intake was associated with increased risk of T2D in all categories of egg intake (HR(95% CI) by category: <1/month (Ref); 1-3/month: 1.12(1.06, 1.20); 1/week: 1.28(1.21, 1.36); 2-4/week: 1.54(1.45, 1.64); 5-6/week: 1.79(1.61, 2.00); ≥1/day: 1.58(1.42, 1.77), p-trend<0.001) (Table 16). After multivariate adjustment, results were largely attenuated (HR(95% CI) by category: <1/month (Ref); 1-3/month: 1.02(0.96, 1.08); 1/week: 1.02(0.96, 1.08); 2-4/week: 1.08(1.01, 1.15); 5-6/week: 1.18(1.06, 1.32); ≥1/day: 1.03(0.92, 1.16), p-trend=0.03) (Table 16). Each increase of one egg per day was associated with an increased risk of T2D of (HR: 1.09, 95% CI: (1.01, 1.18)) (Table 16). We found no significant interactions for age, BMI, physical activity, smoking or family history of T2D (Table 17). Participants who did not have hypertension during follow-up had a somewhat higher risk of T2D with increasing egg intake compared to participants who did not currently have hypertension (p-interaction<0.001). Participants who did not currently have high blood cholesterol had a trend towards increased risk of T2D with increasing egg intake while participants reporting high blood cholesterol during follow-up did not (p-interaction<0.001). Similarly, patients who were not taking statins had a trend towards increased risk of T2D with increasing egg intake, but this was not found among those who reported taking statins (p-interaction=0.001). These results were similar in each of the three cohorts.

Table 15. Age-Standardized Characteristics of Participants in NHSI, NHSII and HPFS in 1994

	Whole Egg Intake								
	Nurses' Health Study I			Nurses' Health Study II			Health Professionals Follow-Up Study		
Characteristics	**0/month**	**2-4/week**	**≥1/day**	**0/month**	**2-4/week**	**≥1/day**	**0/month**	**2-4/week**	**≥1/day**
Participants, No	4,013	15,054	1,251	16,427	11,672	373	6,579	8,113	1,239
Age, mean, y	60.9	59.7	59.5	40.3	40.6	41.1	60.2	61.5	61.8
Body Mass Index*, mean, kg/m^2	25.4	26.8	27.1	24.9	26.6	27.4	25.3	26.4	26.1
Physical Activity, mean, Met h/wk	20.4	19.6	19.6	24.6	18.9	21.9	38.6	35.0	36.9
White, %	98	98	98	96	96	94	95	95	95
Smoking Status									
Never smoker, %	40	47	42	65	67	66	54	48	43
Past smoker, %	44	40	43	25	22	21	42	43	43
Current smoker, %	17	12	15	10	11	13	4	9	14
Postmenopausal, %	90	89	90	8	7	8			
Current menopausal hormone use, %	36	39	39	6	5	4			
Current oral contraceptive use, %				9	7	8			
Family history of diabetes mellitus, %	28	28	32	16	17	19	15	14	13
High blood pressure, %	40	40	42	9	10	16	36	33	29
High blood cholesterol, %	50	45	44	20	16	15	49	33	29
Current multivitamin use, %	43	46	48	59	61	63	64	59	60
Current statin use, %	23	17	16	4	2	3	13	4	3
Dietary intake, mean									
Total Energy, kcal/d	1,459	1,789	1,877	1,583	2,089	2,203	1,792	2,173	2,348
Alcohol, g/d	5.9	6.5	6.7	3.1	3.5	2.9	9.3	12.2	11.7
Bacon, servings/d	0.0	0.1	0.2	0.0	0.1	0.2	0.0	0.2	0.3
Red meat, servings/d	0.9	1.0	1.0	0.5	0.9	0.9	0.5	0.9	1.1
Other processed meat, servings/d	0.1	0.2	0.3	0.1	0.2	0.3	0.1	0.3	0.3
Refined carbohydrates, servings/d	1.0	1.3	1.2	0.8	1.2	1.4	0.8	1.1	1.3
Potatoes, servings/d	0.7	0.8	0.8	0.4	0.6	0.6	0.5	0.6	0.7
Whole milk, servings/d	0.1	0.2	0.3	0.0	0.1	0.2	0.0	0.2	0.3
Coffee, servings/d	2.2	2.4	2.5	1.5	1.6	1.6	1.6	2.1	2.4
Juice, servings/d	0.5	0.5	0.5	0.6	0.8	0.8	0.8	0.8	0.8
Sugar-sweetened beverages, servings /d	0.3	0.3	0.3	0.4	0.6	0.6	0.3	0.4	0.4
Dietary cholesterol, mg/d	186	365	638	173	329	534	191	354	611

*Body mass index calculated as weight in kilograms divided by height in meters squared

Abbreviations: Number (No), Year (y), week (wk), servings (serv), ounces (oz), grams (g)

Table 16. Risk of Incident Type 2 Diabetes According to Categories of Whole Egg Consumption in NHSI, NHSII and HPFS

	Frequency of Consumption Categories						p-value for trend*	HR (95% CI) for a 1 serving per day increase
	<1/month	1-3/month	1/week	2-4/week	5-6/week	≥1/day		
Nurses' Health I Study								
Cases/ Person years, No.	403/101,591	943/239,584	3,529/801,118	2,359/557,457	246/58,218	248/60,136		
Age adjusted model 1	1	0.99 (0.88, 1.12)	1.07 (0.96, 1.18)	1.31 (1.17, 1.45)	1.51 (1.29, 1.78)	1.41 (1.20, 1.66)	<0.001	1.55 (1.40, 1.72)
Multivariable model 2†	1	1.01 (0.90, 1.14)	0.98 (0.89, 1.09)	1.11 (1.00, 1.24)	1.26 (1.07, 1.48)	1.09 (0.92, 1.28)	0.001	1.19 (1.07, 1.25)
Multivariable model 3c	1	1.00 (0.88, 1.12)	0.94 (0.84, 1.05)	1.04 (0.93, 1.16)	1.15 (0.98, 1.36)	1.03 (0.87, 1.21)	0.07	1.11 (0.99, 1.24)
Nurses' Health II Study								
Cases/ Person years, No.	721/314,611	1,559/620,831	2,148/637,282	804/258,040	71/16,852	42/10,009		
Age adjusted model 1	1	1.16 (1.06, 1.27)	1.40 (1.29, 1.53)	1.74 (1.57, 1.93)	2.25 (1.76, 2.88)	2.19 (1.60, 2.99)	<0.001	2.86 (2.44, 3.34)
Multivariable model 2†	1	1.04 (0.95, 1.13)	1.14 (1.04, 1.24)	1.21 (1.09, 1.35)	1.35 (1.05, 1.73)	1.20 (0.87, 1.64)	<0.001	1.45 (1.23, 1.71)
Multivariable model 3‡	1	1.01 (0.92, 1.10)	1.07 (0.97, 1.17)	1.09 (0.98, 1.22)	1.19 (0.92, 1.54)	1.01 (0.73, 1.40)	0.07	1.19 (0.99, 1.42)
Health Professionals Follow-up Study								
Cases/ Person years, No.	415/132,295	682/190,945	1,246/288,898	899/219,283	120/27,960	135/35,481		
Age adjusted model 1	1	1.19 (1.05, 1.35)	1.34 (1.20, 1.50)	1.53 (1.35, 1.72)	1.71 (1.39, 2.10)	1.39 (1.14, 1.70)	<0.001	1.36 (1.21, 1.53)
Multivariable model 2†	1	1.12 (0.99, 1.27)	1.18 (1.05, 1.32)	1.28 (1.14, 1.45)	1.40 (1.13, 1.73)	1.18 (0.96, 1.44)	0.006	1.18 (1.05, 1.33)
Multivariable model 3‡	1	1.07 (0.94, 1.21)	1.07 (0.94, 1.20)	1.11 (0.97, 1.27)	1.19 (0.95, 1.49)	1.02 (0.82, 1.26)	0.67	1.03 (0.90, 1.18)
Pooled Results								
Cases/ Person years, No.	1,539/548,497	3,184/1,051,360	6,923/1,727,298	4,062/1,034,780	437/103,030	425/105,626		
Age adjusted model 1	1	1.12 (1.06, 1.20)	1.28 (1.21, 1.36)	1.54 (1.45, 1.64)	1.79 (1.61, 2.00)	1.58 (1.42, 1.77)	<0.001	1.66 (1.55, 1.77)
Multivariable model 2†	1	1.06 (0.99, 1.12)	1.10 (1.04, 1.17)	1.21 (1.14, 1.29)	1.36 (1.22, 1.52)	1.16 (1.04, 1.30)	<0.001	1.24 (1.16, 1.33)
Multivariable model 3‡	1	1.02 (0.96, 1.08)	1.02 (0.96, 1.08)	1.08 (1.01, 1.15)	1.18 (1.06, 1.32)	1.03 (0.92, 1.16)	0.03	1.09 (1.01, 1.18)

*P-values for trend based on continuous egg variable derived from the median egg intake in each category of consumption

†Multivariable model 2 includes: age (months), sex (male, female), smoking status (never, former, current), BMI (kg/m^2: categorical <21, 21-<23, 23-<25, 25-<27, 27-<30, 30-<35, 35-<40, 40+), physical activity (MET-hours/week: categorical <3, 3-<9, 9-<18, 18-<27, 27+), oral contraceptive use (never, former, current), post-menopausal hormone use (premenopausal, never, former, current), race (Caucasian, other), family history of T2D (yes, no), high blood pressure at baseline (yes, no), high blood cholesterol at baseline (yes, no), alcohol intake (servings/d), multivitamin use (yes, no)

‡Multivariable model 3 includes: model 2 with total calories (per day: quintiles), whole milk (categorical: servings/d), bacon (categorical: servings/d), red meat (categorical: servings/d), other processed meat (categorical: servings/d), refined carbohydrates (categorical: servings/d), potatoes (categorical: servings/d), coffee (categorical: servings/d), juice (categorical: servings/d), sugar-sweetened beverages (categorical: servings/d)

Table 17. Stratified Analysis of Risk of Incident Type 2 Diabetes According to Categories of Whole Egg Consumption in NHSI, NHSII and HPFS: Pooled Results*

Variable	Cases/Person Years	Frequency of Consumption Categories						p-value for trend†
		<1/month	1-3/month	1/week	2-4/week	5-6/week	≥1/day	
Age < 65	11,584/3.686,096	1	1.03 (0.96, 1.11)	1.04 (0.97, 1.11)	1.13 (1.05, 1.22)	1.23 (1.07, 1.40)	1.07 (0.93, 1.22)	0.009
Age ≥ 65	4,986/884,494	1	0.98 (0.86, 1.10)	0.96 (0.85, 1.07)	0.99 (0.87, 1.11)	1.08 (0.87, 1.33)	0.94 (0.77, 1.16)	0.92
BMI < 25	2,480/2,410,378	1	0.95 (0.81, 1.11)	0.93 (0.80, 1.07)	1.07 (0.91, 1.25)	1.09 (0.81, 1.47)	1.00 (0.74, 1.33)	0.30
BMI ≥ 25	14,090/2,160,212	1	1.03 (0.97, 1.11)	1.04 (0.98, 1.11)	1.09 (1.02, 1.18)	1.21 (1.07, 1.37)	1.03 (0.91, 1.17)	0.05
≥ 10 MET/wk PA	9,374/1,995,971	1	1.02 (0.94, 1.11)	0.99 (0.92, 1.08)	1.08 (0.99, 1.18)	1.21 (1.04, 1.41)	0.96 (0.83, 1.13)	0.25
< 10 MET/wk PA	7,196/2,574,619	1	1.02 (0.93, 1.12)	1.04 (0.95, 1.13)	1.07 (0.97, 1.18)	1.18 (0.99, 1.41)	1.15 (0.97, 1.37)	0.03
Non smoker	8,434/2,624,017	1	1.01 (0.93, 1.10)	1.04 (0.96, 1.13)	1.11 (1.02, 1.22)	1.29 (1.10, 1.51)	1.09 (0.91, 1.29)	0.005
Ever smoker	8136/1,946,573	1	1.03 (0.94, 1.13)	0.98 (0.90, 1.06)	1.04 (0.95, 1.15)	1.11 (0.94, 1.30)	1.00 (0.85, 1.17)	0.58
No hypertension	5,974/3,255,712	1	0.93 (0.84, 1.03)	0.98 (0.89, 1.08)	1.07 (0.96, 1.19)	1.17 (0.98, 1.40)	1.10 (0.92, 1.32)	0.004
Hypertension	10,596/1,314,878	1	1.08 (1.00, 1.17)	1.05 (0.97, 1.13)	1.12 (1.03, 1.22)	1.22 (1.05, 1.42)	1.02 (0.88, 1.19)	0.20
Normal blood cholesterol	7,136/3,081,197	1	0.98 (0.88, 1.08)	1.04 (0.94, 1.14)	1.15 (1.04, 1.28)	1.21 (1.03, 1.42)	1.07 (0.90, 1.26)	0.006
High blood cholesterol	9,434/1,489,393	1	1.05 (0.97, 1.14)	1.01 (0.94, 1.09)	1.05 (0.96, 1.14)	1.15 (0.97, 1.36)	1.02 (0.86, 1.21)	0.55
No statin use	13,068/4,179,438	1	1.02 (0.95, 1.10)	1.04 (0.97, 1.11)	1.14 (1.06, 1.22)	1.25 (1.11, 1.42)	1.10 (0.97, 1.24)	<0.001
Statin use	3,502/391,152	1	1.05 (0.92, 1.19)	1.01 (0.90, 1.14)	0.97 (0.84, 1.12)	0.97 (0.70, 1.34)	0.86 (0.62, 1.20)	0.20
No family history	8,681/3,311,468	1	1.03 (0.95, 1.13)	1.05 (0.97, 1.14)	1.09 (1.00, 1.20)	1.17 (1.00, 1.37)	1.12 (0.96, 1.30)	0.03
Family history of T2D	7,889/1,259,122	1	1.00 (0.92, 1.10)	0.99 (0.91, 1.08)	1.09 (0.99, 1.20)	1.18 (1.00, 1.40)	0.95 (0.79, 1.13)	0.27

*All results use multivariable model 3 and include: age (months), sex (male, female), smoking status (never, former, current), BMI (kg/m^2: categorical <21, 21-<23, 23-<25, 25-<27, 27-<30, 30-<35, 35-<40, 40+), physical activity (MET-hours/week: categorical <3, 3-<9, 9-<18, 18-<27, 27+), oral contraceptive use (never, former, current), post-menopausal hormone use (premenopausal, never, former, current), race (Caucasian, other), family history of T2D (yes, no), high blood pressure at baseline (yes, no), high blood cholesterol at baseline (yes, no), statin use at baseline (yes, no: only available for HPFS), alcohol intake (servings/d), multivitamin use (yes, no), total calories (per day: quintiles), whole milk (categorical: servings/d), bacon (categorical: servings/d), red meat (categorical: servings/d), other processed meat (categorical: servings/d), refined carbohydrates (categorical: servings/d), potatoes (categorical: servings/d), coffee (categorical: servings/d), juice (categorical: servings/d), sugar-sweetened beverages (categorical: servings/d)

†P-values for trend based on continuous egg variable derived from the median egg intake in each category of consumption

‡P-values for interaction calculated using the likelihood ratio test

Table 17 (Continued)

Variable	Cases/Person Years	p-value for trend[†]	HR (95% CI) for a 1 serving per day increase	p-int.[‡]
Age < 65	11,584/3.686,096	0.009	1.13 (1.03, 1.24)	0.11
Age ≥ 65	4,986/884,494	0.92	1.01 (0.88, 1.16)	
BMI < 25	2,480/2,410,378	0.30	1.11 (0.91, 1.35)	0.61
BMI ≥ 25	14,090/2,160,212	0.05	1.09 (1.00, 1.18)	
≥ 10 MET/wk PA	9,374/1,995,971	0.25	1.06 (0.96, 1.18)	0.16
< 10 MET/wk PA	7,196/2,574,619	0.03	1.14 (1.01, 1.28)	
Non smoker	8,434/2,624,017	0.005	1.18 (1.05, 1.31)	0.50
Ever smoker	8136/1,946,573	0.58	1.03 (0.93, 1.15)	
No hypertension	5,974/3,255,712	0.004	1.20 (1.06, 1.35)	<0.001
Hypertension	10,596/1,314,878	0.20	1.07 (0.97, 1.18)	
Normal blood cholesterol	7,136/3,081,197	0.006	1.16 (1.04, 1.29)	<0.001
High blood cholesterol	9,434/1,489,393	0.55	1.03 (0.93, 1.16)	
No statin use	13,068/4,179,438	<0.001	1.16 (1.07, 1.26)	0.001
Statin use	3,502/391,152	0.20	0.87 (0.71, 1.07)	
No family history	8,681/3,311,468	0.03	1.12 (1.01, 1.24)	0.09
Family history of T2D	7,889/1,259,122	0.27	1.07 (0.95, 1.20)	

[*]All results use multivariable model 3 and include: age (months), sex (male, female), smoking status (never, former, current), BMI (kg/m^2: categorical <21, 21-<23, 23-<25, 25-<27, 27-<30, 30-<35, 35-<40, 40+), physical activity (MET-hours/week: categorical <3, 3-<9, 9-<18, 18-<27, 27+), oral contraceptive use (never, former, current), post-menopausal hormone use (premenopausal, never, former, current), race (Caucasian, other), family history of T2D (yes, no), high blood pressure at baseline (yes, no), high blood cholesterol at baseline (yes, no), statin use at baseline (yes, no: only available for HPFS), alcohol intake (servings/d), multivitamin use (yes, no), total calories (per day: quintiles), whole milk (categorical: servings/d), bacon (categorical: servings/d), red meat (categorical: servings/d), other processed meat (categorical: servings/d), refined carbohydrates (categorical: servings/d), potatoes (categorical: servings/d), coffee (categorical: servings/d), juice (categorical: servings/d), sugar-sweetened beverages (categorical: servings/d)

[†]P-values for trend based on continuous egg variable derived from the median egg intake in each category of consumption

[‡]P-values for interaction calculated using the likelihood ratio test

When we examined the effect of substituting eggs by other food groups on the risk of T2D we found that substituting eggs with legumes (HR: 0.91, 95% CI: (0.84, 0.98)), low fat dairy (HR: 0.88, 95% CI: (0.83, 0.93)), nuts (HR: 0.86, 95% CI: (0.80, 0.92)) and whole grains (HR: 0.82, 95% CI: (0.77, 0.87)) significantly decreased the risk of T2D. Substituting eggs with red and processed meats increased the risk of T2D (HR: 1.09, 95% CI: (1.02, 1.16)) (Figure 6).

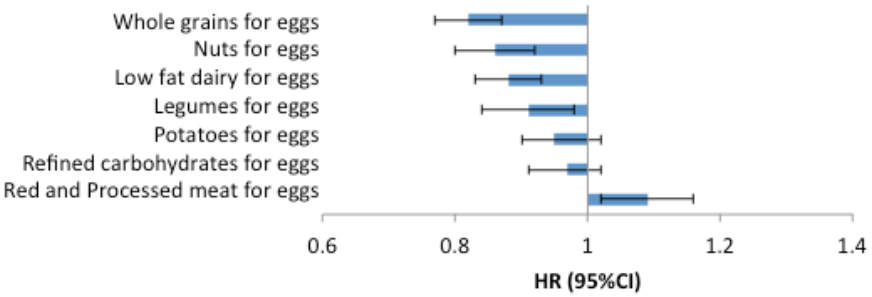


Figure 6. Hazard ratios and 95% CI's for incident T2D associated with replacement of eggs with other food groups. Adjusted for: age (months), sex (male, female), smoking status (never, former, current), BMI (kg/m^2: categorical <23, 23-<25, 25-<27, 27-<30, 30-<35, 35-<40, 40+), physical activity (MET-hours/week: categorical <3, 3-<9, 9-<18, 18-<27, 27+), oral contraceptive use (never, former, current), post-menopausal hormone use (premenopausal, never, former, current), race (Caucasian, other), family history of MI (yes, no), baseline high blood cholesterol (yes, no), baseline high blood pressure (yes, no) statin use (yes, no), Alcohol intake (servings/d), multivitamin use (yes, no), total calories (per day: quintiles)

In sensitivity analyses, we examined the joint effect of eggs and BMI on risk of T2D. We found an overall higher risk across categories of BMI compared to categories of egg intake (p-value for interaction=0.08) (Figure 7, Table 18). When we examined the risk of incident T2D according to seven categories of intake, we found a risk of (HR: 1.07, 95% CI: (0.84, 1.37)) in participants consuming at least two eggs per day. This was similar to the category of one egg per day (HR: 1.02, 95% CI: (0.91, 1.16)) (Table 19).

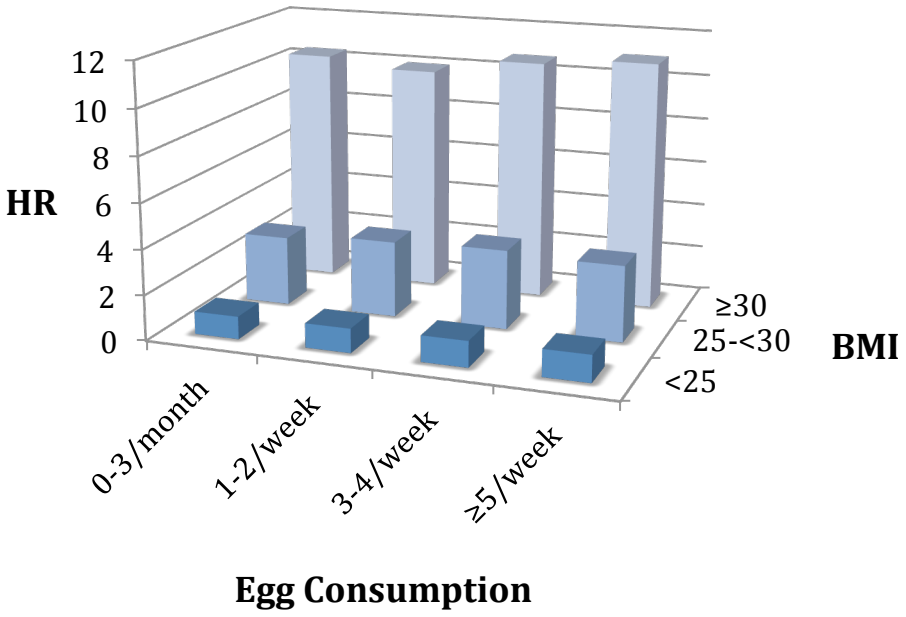


Figure 7. Risk of Incident Type 2 Diabetes According to Joint Effects of Egg Consumption and BMI. Adjusted for age (months), sex (male, female), smoking status (never, former, current), physical activity (MET-hours/week: categorical <3, 3-<9, 9-<18, 18-<27, 27+), oral contraceptive use (never, former, current), post-menopausal hormone use (premenopausal, never, former, current), race (Caucasian, other), family history of T2D (yes, no), high blood pressure at baseline (yes, no), high blood cholesterol at baseline (yes, no), statin use at baseline (yes, no: only available for HPFS), alcohol intake (servings/d), multivitamin use (yes, no), total calories (per day: quintiles), whole milk (categorical: servings/d), bacon (categorical: servings/d), red meat (categorical: servings/d), other processed meat (categorical: servings/d), refined carbohydrates (categorical: servings/d), potatoes (categorical: servings/d), coffee (categorical: servings/d), juice (categorical: servings/d), sugar-sweetened beverages (categorical: servings/d

Table 18. Risk of Incident Type 2 Diabetes According to the Joint Effects of Egg Consumption and BMI*

BMI		Frequency of Egg Consumption				p-trend†	HR (95% CI) for a 1 serving per day increase
		≤3/month	1/week	2-4/week	≥5/week		
<25 kg/m^2	Cases/ Person years, No	614/832,900	776/764,294	417/420,149	89/83,198		
	HR (95% CI)	1	1.09 (0.98, 1.22)	1.16 (1.02, 1.31)	1.22 (0.97, 1.53)	0.87	1.02 (0.79, 1.32)
25-<30 kg/m^2	Cases/ Person years, No	1,349/451,497	2,020/519,969	1,067/284,949	208/55,908		
	HR (95% CI)	3.13 (2.84, 3.44)	3.42 (3.12, 3.75)	3.62 (3.27, 4.01)	3.51 (2.98, 4.13)	0.47	1.06 (0.90, 1.24)
≥30 kg/m^2	Cases/ Person years, No	2,555/234,018	3,702/285,830	1,995/154,154	417/28,921		
	HR (95% CI)	10.61 (9.70, 11.62)	10.25 (9.39, 11.20)	10.88 (9.90, 11.97)	11.30 (9.91, 12.89)	0.002	1.20 (1.07, 1.35)

*All results use multivariable model 3 and include: age (months), sex (male, female), smoking status (never, former, current), physical activity (MET-hours/week: categorical <3, 3-<9, 9-<18, 18-<27, 27+), oral contraceptive use (never, former, current), post-menopausal hormone use (premenopausal, never, former, current), race (Caucasian, other), family history of T2D (yes, no), high blood pressure at baseline (yes, no), high blood cholesterol at baseline (yes, no), Alcohol intake (servings/d), multivitamin use (yes, no), total calories (per day: quintiles), whole milk (categorical: servings/d), bacon (categorical: servings/d), red meat (categorical: servings/d), other processed meat (categorical: servings/d), refined carbohydrates (categorical: servings/d), potatoes (categorical: servings/d), coffee (categorical: servings/d), juice (categorical: servings/d), sugar-sweetened beverages (categorical: servings/d)

†P-values for trend based on continuous egg variable derived from the median egg intake in each category of consumption

Table 19. Risk of Incident Type 2 Diabetes According to Seven Categories of Egg Consumption*

	Frequency of Consumption Categories							p-trend†	HR (95% CI) for a 1 serving per day increase
	<1/month	1-3/month	1/week	2-4/week	5-6/week	1/day	≥2/day		
Cases/Person-years, No.	1,539/548,497	3,184/1,051,360	6,923/1,727,298	4,062/1,034,780	437/103,030	353/86,612	72/19,015		
NHS	1	1.00 (0.88, 1.12)	0.94 (0.84, 1.05)	1.04 (0.93, 1.16)	1.15 (0.98, 1.36)	1.00 (0.84, 1.19)	1.23 (0.86, 1.75)	0.03	1.12 (1.01, 1.24)
NHSII	1	1.01 (0.92, 1.10)	1.07 (0.97, 1.17)	1.09 (0.98, 1.22)	1.19 (0.92, 1.54)	1.08 (0.77, 1.51)	0.55 (0.17, 1.73)	0.18	1.12 (0.95, 1.31)
HPFS	1	1.07 (0.94, 1.21)	1.07 (0.94, 1.20)	1.11 (0.97, 1.27)	1.19 (0.95, 1.49)	1.00 (0.79, 1.27)	1.07 (0.74, 1.55)	0.61	1.03 (0.91, 1.16)
Pooled Results	1	1.02 (0.96, 1.08)	1.02 (0.96, 1.08)	1.08 (1.01, 1.15)	1.18 (1.06, 1.32)	1.02 (0.91, 1.16)	1.07 (0.84, 1.37)	0.03	1.08 (1.01, 1.16)

*All results are from Multivariable Model 3 and include: age (months), sex (male, female), smoking status (never, former, current), BMI (kg/m^2: categorical <21, 21-<23, 23-<25, 25-<27, 27-<30, 30-<35, 35-<40, 40+), physical activity (MET-hours/week: categorical <3, 3-<9, 9-<18, 18-<27, 27+), oral contraceptive use (never, former, current), post-menopausal hormone use (premenopausal, never, former, current), race (Caucasian, other), family history of T2D (yes, no), incident high blood pressure (yes, no), incident high blood cholesterol (yes, no), Alcohol intake (servings/d), multivitamin use (yes, no), total calories (per day: quintiles), whole milk (categorical: servings/d), bacon (categorical: servings/d), red meat (categorical: servings/d), other processed meat (categorical: servings/d), poultry (categorical: servings/d), fish (categorical: servings/d), refined carbohydrates (categorical: servings/d), potatoes (categorical: servings/d), coffee (categorical: servings/d), juice (categorical: servings/d), sugar-sweetened beverages (categorical: servings/d)

†P-values for trend based on continuous egg variable derived from the median egg intake in each category of consumption

Results were attenuated when we examined eggs from all sources including mixed dishes (HR for ≥2 egg/day: 1.05, 95% CI: (0.84, 1.30)) (Table 20). When we examined the risk of T2D according to egg intake additionally adjusted for dietary cholesterol, we found that results were attenuated, and no longer significant (HR for an increase of 1 egg/day: 1.02, 95% CI: (0.93, 1.13)) (Table 21).We also examined dietary cholesterol separately from egg intake. Dietary cholesterol was updated using the cumulative average method. We found a significantly increased risk of T2D across Q2 to Q5 of dietary cholesterol (HR(95% CI) by category: Q1 (Ref); Q2: 1.16(1.09, 1.22); Q3: 1.19(1.12, 1.25); Q4: 1.23(1.26, 1.30); Q5: 1.33(1.25, 1.42)) (Table 22). When we examined the risk of T2D across quintiles of dietary cholesterol adjusting for egg intake, we saw a slight attenuation of risk across Q1-Q5 (HR(95% CI) by category: Q1 (Ref); Q2: 1.15(1.08, 1.22); Q3: 1.17(1.09, 1.24); Q4: 1.20(1.11, 1.28); Q5: 1.26(1.16, 1.37)) (Table 23). When we further adjusted for red and processed meats in the model, we saw a larger attenuation of results, suggesting contribution of red and processed meats to the increased risk of T2D associated with dietary cholesterol (HR(95% CI) by category: Q1 (Ref); Q2: 1.12(1.05, 1.19); Q3: 1.12(1.05, 1.20); Q4: 1.13(1.05, 1.22); Q5: 1.17(1.08, 1.28)).

We did not find any clinically meaningful changes in egg intake after diagnosis with high blood pressure, high blood cholesterol or beginning statin use. When we stopped updating dietary information after diagnosis with one of these possible intermediate outcomes, we did not find any changes in risk of T2D (HR for 1 egg/day: 1.09, 95% CI: (0.97, 1.23)). Using a simple update of diet rather than cumulative average did not significantly change results (HR for 1 egg/day: 1.09, 95% CI: (0.98, 1.21)); however, using baseline diet only slightly attenuated the results, likely due to the long follow-up period between baseline diet and development of T2D (HR for 1 egg/day: 1.04, 95% CI: (0.95, 1.14)).

Table 20. Risk of Incident Type 2 Diabetes According to Categories of Total Egg Consumption, Including Eggs in Mixed Foods*

	Frequency of Consumption Categories							p-trend†	HR (95% CI) for a 1 serving per day increase
	<1/month	1-3/month	1/week	2-4/week	5-6/week	1/day	≥2/day		
Cases/Person-years, No.	808/300,339	2,481/848,173	7,493/1,997,155	4,653/1,158,450	568/122,440	469/108,537	98/24,496		
NHS	1	0.92 (0.79, 1.06)	0.89 (0.77, 1.02)	0.95 (0.82, 1.09)	1.05 (0.88, 1.25)	0.95 (0.78, 1.15)	1.13 (0.80, 1.61)	0.04	1.11 (1.01, 1.22)
NHSII	1	0.93 (0.82, 1.05)	0.92 (0.82, 1.04)	1.00 (0.88, 1.14)	1.09 (0.85, 1.39)	0.99 (0.72, 1.36)	0.52 (0.16, 1.65)	0.25	1.10 (0.94, 1.28)
HPFS	1	1.11 (0.94, 1.30)	1.11 (0.95, 1.29)	1.09 (0.93, 1.28)	1.04 (0.83, 1.29)	1.14 (0.93, 1.40)	1.15 (0.85, 1.56)	0.64	1.02 (0.93, 1.13)
Pooled Results	1	0.97 (0.90, 1.05)	0.96 (0.89, 1.03)	1.01 (0.93, 1.09)	1.06 (0.94, 1.18)	1.01 (0.89, 1.14)	1.05 (0.84, 1.30)	0.06	1.06 (1.00, 1.13)

*All results are from Multivariable Model 3 and include: age (months), sex (male, female), smoking status (never, former, current), BMI (kg/m^2: categorical <21, 21-<23, 23-<25, 25-<27, 27-<30, 30-<35, 35-<40, 40+), physical activity (MET-hours/week: categorical <3, 3-<9, 9-<18, 18-<27, 27+), oral contraceptive use (never, former, current), post-menopausal hormone use (premenopausal, never, former, current), race (Caucasian, other), family history of T2D (yes, no), incident high blood pressure (yes, no), incident high blood cholesterol (yes, no), Alcohol intake (servings/d), multivitamin use (yes, no), total calories (per day: quintiles), whole milk (categorical: servings/d), bacon (categorical: servings/d), red meat (categorical: servings/d), other processed meat (categorical: servings/d), poultry (categorical: servings/d), fish (categorical: servings/d), refined carbohydrates (categorical: servings/d), potatoes (categorical: servings/d), coffee (categorical: servings/d), juice (categorical: servings/d), sugar-sweetened beverages (categorical: servings/d)

†P-values for trend based on continuous egg variable derived from the median egg intake in each category of consumption

Table 21. Risk of Incident Type 2 Diabetes According to Egg Intake, Adjusting for Dietary Cholesterol*

	Frequency of Consumption Categories						p- trend†	HR (95% CI) for a 1 egg per day increase
Cohort	<1/month	1-3/month	1/week	2-4/week	5-6/week	≥1/day		
Pooled	1	1.01 (0.94, 1.07)	0.99 (0.92, 1.05)	1.04 (0.96, 1.12)	1.13 (1.00, 1.28)	0.99 (0.87, 1.12)	0.64	1.02 (0.93, 1.13)

*All results are from Multivariable Model 3 and include: dietary cholesterol (quintiles: mg/d), age (months), sex (male, female), smoking status (never, former, current), BMI (kg/m^2: categorical <21, 21-<23, 23-<25, 25-<27, 27-<30, 30-<35, 35-<40, 40+), physical activity (MET-hours/week: categorical <3, 3-<9, 9-<18, 18-<27, 27+), oral contraceptive use (never, former, current), post-menopausal hormone use (premenopausal, never, former, current), race (Caucasian, other), family history of T2D (yes, no), incident high blood pressure (yes, no), incident high blood cholesterol (yes, no), Alcohol intake (servings/d), multivitamin use (yes, no), total calories (per day: quintiles), whole milk (categorical: servings/d), bacon (categorical: servings/d), red meat (categorical: servings/d), other processed meat (categorical: servings/d), poultry (categorical: servings/d), fish (categorical: servings/d), refined carbohydrates (categorical: servings/d), potatoes (categorical: servings/d), coffee (categorical: servings/d), juice (categorical: servings/d), sugar-sweetened beverages (categorical: servings/d)

†P-values for trend based on continuous egg variable derived from the median egg intake in each category of consumption

Table 22. Risk of Incident Type 2 Diabetes According to Quintiles of Dietary Cholesterol*

	Quintiles of Dietary Cholesterol					p- trend†	HR (95% CI) for a 200mg per day increase
	Q1	**Q2**	**Q3**	**Q4**	**Q5**		
Median (mg/d)	155.0	215.0	262.6	311.0	398.2		
Pooled	1	1.16 (1.09, 1.22)	1.19 (1.12, 1.25)	1.23 (1.26, 1.30)	1.33 (1.25, 1.42)	<0.001	1.14 (1.10, 1.18)

*All results are from Multivariable Model 3 and include: age (months), sex (male, female), smoking status (never, former, current), BMI (kg/m^2: categorical <21, 21-<23, 23-<25, 25-<27, 27-<30, 30-<35, 35-<40, 40+), physical activity (MET-hours/week: categorical <3, 3-<9, 9-<18, 18-<27, 27+), oral contraceptive use (never, former, current), post-menopausal hormone use (premenopausal, never, former, current), race (Caucasian, other), family history of T2D (yes, no), incident high blood pressure (yes, no), incident high blood cholesterol (yes, no), Alcohol intake (servings/d), multivitamin use (yes, no), total calories (per day: quintiles), whole milk (categorical: servings/d), refined carbohydrates (categorical: servings/d), potatoes (categorical: servings/d), coffee (categorical: servings/d), juice (categorical: servings/d), sugar-sweetened beverages (categorical: servings/d)

†P-values for trend based on continuous dietary cholesterol derived from the median in each quintile

Table 23. Risk of Incident Type 2 Diabetes According to Dietary Cholesterol from Sources Other than Egg Intake*

	Quintiles of Dietary Cholesterol					p- trend†	HR (95% CI) for a 200mg per day increase
	Q1	**Q2**	**Q3**	**Q4**	**Q5**		
Median (mg/d)	155.0	215.0	262.6	311.0	398.2		
Pooled	1	1.15 (1.08, 1.22)	1.17 (1.09, 1.24)	1.20 (1.11, 1.28)	1.26 (1.16, 1.37)	<0.001	1.13 (1.07, 1.19)

*All results are from Multivariable Model 3 and include: egg intake (categorical <1/month, 1-3/month, 1/week, 2-4/week, 5-6/week, ≥1/day), age (months), sex (male, female), smoking status (never, former, current), BMI (kg/m^2: categorical <21, 21-<23, 23-<25, 25-<27, 27-<30, 30-<35, 35-<40, 40+), physical activity (MET-hours/week: categorical <3, 3-<9, 9-<18, 18-<27, 27+), oral contraceptive use (never, former, current), post-menopausal hormone use (premenopausal, never, former, current), race (Caucasian, other), family history of T2D (yes, no), incident high blood pressure (yes, no), incident high blood cholesterol (yes, no), Alcohol intake (servings/d), multivitamin use (yes, no), total calories (per day: quintiles), whole milk (categorical: servings/d), refined carbohydrates (categorical: servings/d), potatoes (categorical: servings/d), coffee (categorical: servings/d), juice (categorical: servings/d), sugar-sweetened beverages (categorical: servings/d)

†P-values for trend based on continuous egg variable derived from the median egg intake in each category of consumption

In our meta-analysis of egg intake and risk of T2D, we identified 583 articles from the literature search. 582 articles were found by searching PubMed and Embase and one additional article was found though a review of the article references. We excluded 567 articles that did not mention both eggs and T2D, leaving 16 articles for full text review (Figure 8). Of these, 10 were excluded. Three were review articles, 2 were commentaries, 2 did not have T2D as the outcome, 2 were not prospective cohorts and 1 had egg intake less than 1 egg per week. A total of 7 studies were included in the meta-analyses, including the current analyses (Table 24). With fixed effects meta-analysis, we observed an increased risk of T2D with an increase of one egg per day (HR: 1.13, 95% CI: (1.07, 1.19)) (Figure 9A). When random effects meta-analysis was used, more weight was given to smaller studies and the results were attenuated and no longer significant (HR: 1.04, 95% CI: (0.88, 1.23)) (Figure 9B).

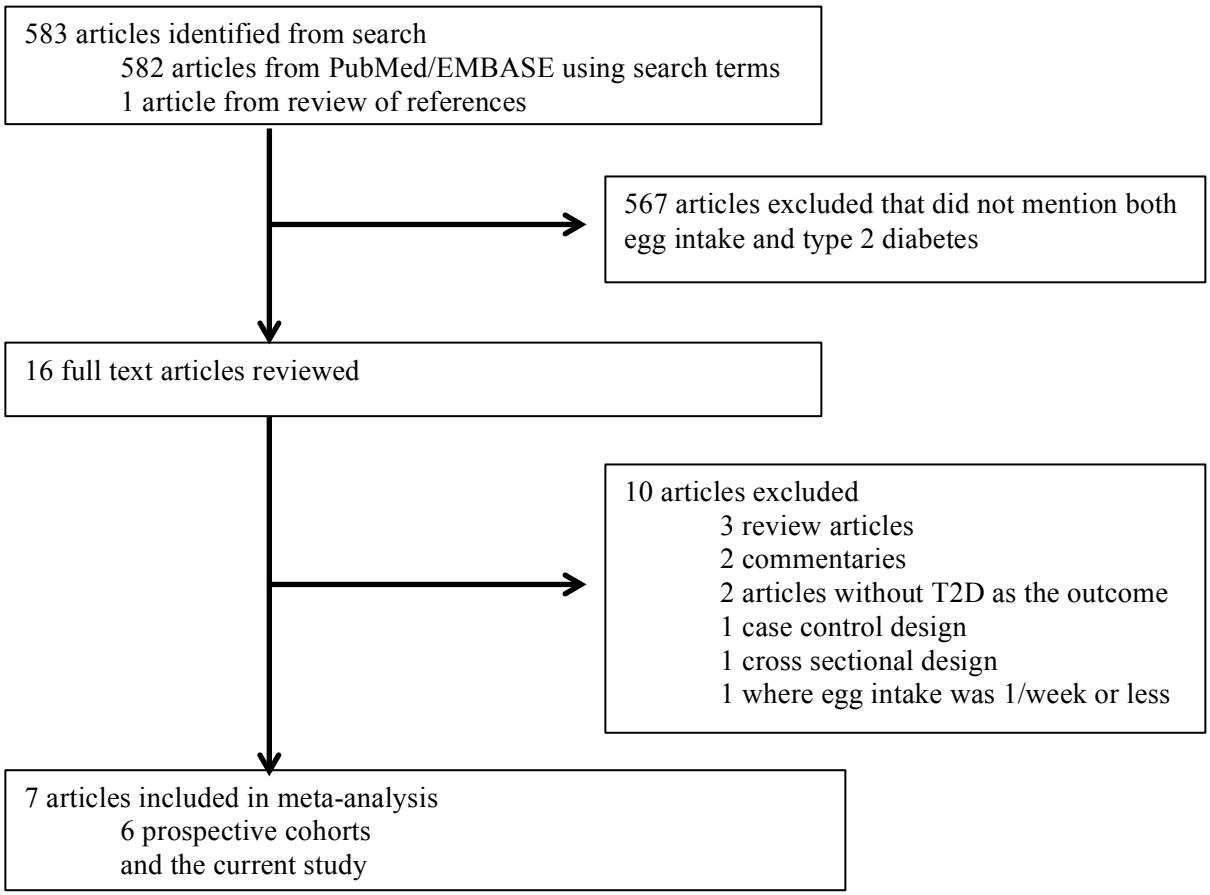


Figure 8. Meta-analysis Search Strategy and Study Selection

Table 24. Meta-analysis Study Characteristics

Study	Study Population	Country	Number of Participants	Age Range	Study Type (Follow-up)	Assessment of Exposure	Categories of Exposure	Number of Cases
Djousse et al. 2009	Physicians' Health Study (PHS); Womens' Health Study (WHS)	USA	20,703 Men; 36,295 Women	Men: 39.7-85.9 y; Women: 38.7-89.9 y	Prospective Cohort (Men: 10.0 y mean; Women: 11.7 y mean)	Semi-quantitative FFQ	0; <1/wk; 1/wk; 2-4/wk; 5-6/wk; ≥7/wk	1,921 Men; 2,112 Women
Djousse et al. 2010	Cardiovascular Health Study (CHS)	USA	3,898 Men and Women	≥65 y	Prospective Cohort (11.3 y mean)	Semi-quantitative FFQ	Never; <1/mo; 1-3/mo; 1-4/wk; Almost daily	142 Men; 171 Women
Djousse et al. 2015	Jackson Heart Study	USA	3,564 African American Men	21-95 y	Prospective Cohort (7.3 y mean)	Semi-quantitative FFQ	<1/mo; 1-3/mo; 1/wk; 2/wk; 3-4/wk; ≥5/wk	531 Men

Table 24 (Continued)

Study	Study Population	Ascertainment of Cases	Statistical Model	Relative Risks (95% CI)	Covariates in Multivariate Model
Djousse et al. 2009	Physicians' Health Study (PHS); Womens' Health Study (WHS)	Self-report validated through telephone interviews, supplemental questionnaires or review of medical records	Cox proportional hazards	Men: 0: 1(Ref); <1/wk: 1.09 (0.87, 1.37); 1/wk: 1.09(0.88, 1.34); 2-4/wk: 1.18(0.95, 1.45); 5-6/wk: 1.46(1.14, 1.86); ≥7/wk: 1.58(1.25, 2.01); Women: 0: 1(Ref); <1/wk: 1.06(0.92, 1.22); 1/wk: 0.97(0.83, 1.12); 2-4/wk: 1.19(1.03, 1.38); 5-6/wk: 1.18(0.88, 1.58); ≥7/wk: 1.77(1.28, 2.43)	Men: age, BMI, smoking, alcohol, exercise ,history of hypercholesterolemia and hypertension, Women: age, BMI, smoking, alcohol, exercise, red meat, energy intake, fruits, vegetables, saturated fatty acids, trans fatty acids, family history of diabetes, history of hypercholesterolemia and hypertension
Djousse et al. 2010	Cardiovascular Health Study (CHS)	Report of new use of insulin or oral hypoglycemia agents, fasting plasma glucose >7.0 mmol/L, or non-fasting plasma glucose >11.1 mmol/L	Cox proportional hazards	Men: Never: 1(Ref); <1/mo: 0.95(0.45, 2.01); 1-3/mo: 1.14(0.60, 2.5); 1-4/wk: 0.96(0.50, 1.82); Almost daily: 1.81(0.77, 4.22); Women: Never: 1(Ref); <1/mo: 0.77(0.43, 1.38); 1-3/mo: 0.73(0.47, 1.14); 1-4/wk: 0.76(0.47, 1.23); Almost daily: 0.38(0.10, 1.37)	age, race, BMI, smoking, alcohol, physical activity, cereal-fiber intake, field center
Djousse et al. 2015	Jackson Heart Study	Fasting glucose ≥126 mg/dL, A1c ≥6.5% or use of insulin or oral hypoglycemic medication	Cox proportional hazards	<1/mo(Ref); 1-3/mo: 0.88(0.65, 1.19); 1/wk: 0.94(0.68, 1.30); 2/wk: 0.91(0.66, 1.25); 3-4/wk: 1.11(0.81, 1.52); ≥5/wk: 1.17(0.81, 1.70)	Age, sex, smoking, alcohol, BMI, physical activity score, education, energy intake, red meat and bacon, fiber, dietary magnesium, fruit/vegetables, trans fat, waist circumference, history of hypertension and history of CVD

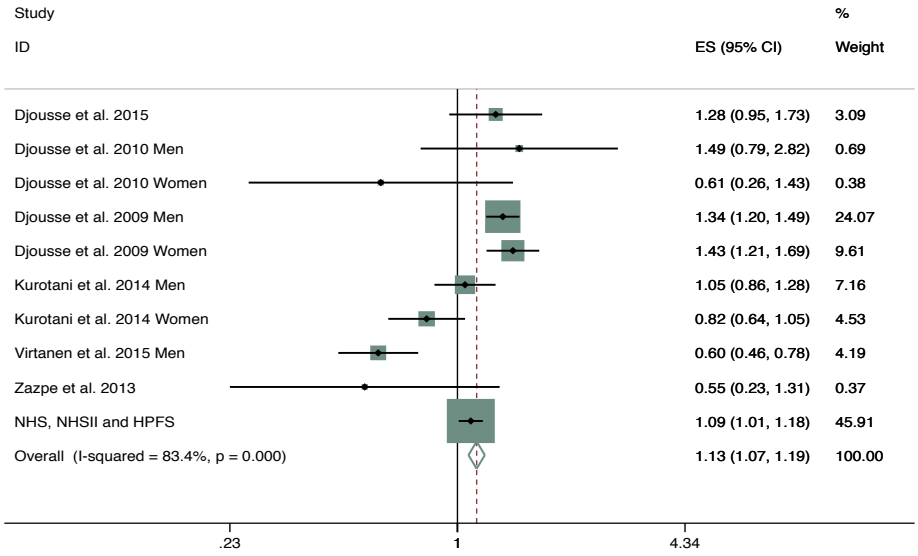
Table 24 (Continued)

Study	Study Population	Country	Number of Participants	Age Range	Study Type (Follow-up)	Assessment of Exposure	Categories of Exposure	Number of Cases
Kurotani et al. 2014	Japan Public Health Center-based Prospective Study	Japan	27,248 Men and 36,218 Women	40-69 y	Prospective Cohort (5 y)	Semi-quantitative FFQ	Quartiles: Men: 7.7g/d; 19.4g/d; 32.6g/d; 55.0g/d; Women: 6.9g/d; 17.5g/d; 29.4g/d; 50.3g/d	672 Men; 493 Women
Virtanen et al. 2015	Kuopio Ischaemic Heart Disease Risk Factor Study	Finland	2,332 Men	42-60 y	Prospective Cohort (19.3 y)	Guided 4-day food record	Quartiles: <14g/d; 14-26g/d; 27-45g/d; >45g/d	432
Zazpe et al. 2013	The Sun project	Spain	15,956 men and women	20-90 y	Prospective Cohort (6.6 y mean)	Semi-quantitative FFQ	<1/week; 1/week; 2-4/week; >4/week	91

Table 24 (Continued)

Study	Study Population	Ascertainment of Cases	Statistical Model	Relative Risks (95% CI)	Covariates in Multivariate Model
Kurotani et al. 2014	Japan Public Health Center-based Prospective Study	Self-report disease history questionnaire	Multivariate logistic regression	Men: Q1(Ref); Q2: 0.93(0.74, 1.15); Q3: 0.93 (0.74, 1.16); Q4: 1.06 (0.85, 1.32); Women: Q1(Ref); Q2:1.01(0.79, 1.29); Q3: 0.94(0.73, 1.21); Q4: 0.82(0.63, 1.06)	Age, public health center area, BMI, smoking status, alcohol consumption, total physical activity levels, history of hypertension, family history of diabetes, Mg intake, Ca intake, coffee, rice, fish, shellfish, meat, vegetables, soft drinks and total energy
Virtanen et al. 2015	Kuopio Ischaemic Heart Disease Risk Factor Study	Self-reported, fasting plasma glucose ≥7.0 mmol/L or 2-hr OGTT plasma glucose ≥11.1 mmol/L	Cox proportional hazards	<14g/d: 1(Ref); 14-26g/d: 0.90(0.69, 1.16); 27-45g/d: 0.59(0.44, 0.80); >45g/d: 0.55(0.38, 0.79)	Age, examination year, energy intake, BMI, family history of type 2 diabetes, hypertension, smoking, education years, leisure-time physical activity, serum long-chain omega-3 PUFAs, alcohol, linoleic acid, fiber, fruit, berries, vegetables
Zazpe et al. 2013	The Sun project	Self-report validated through supplemental questionnaires and medical record review	Multivariate logistic regression	<1/week: 1(Ref); 1/week: 0.9 (0.4, 1.8); 2-4/week: 0.6 (0.3, 1.2); >4/week: 0.7 (0.3, 1.7)	Age, sex, total energy intake, adherence to Mediterranean food pattern, alcohol, baseline BMI, smoking status, leisure time physical activity, family history of T2D, self-reported hypertension, hypercholesterolemia and

A.



B.

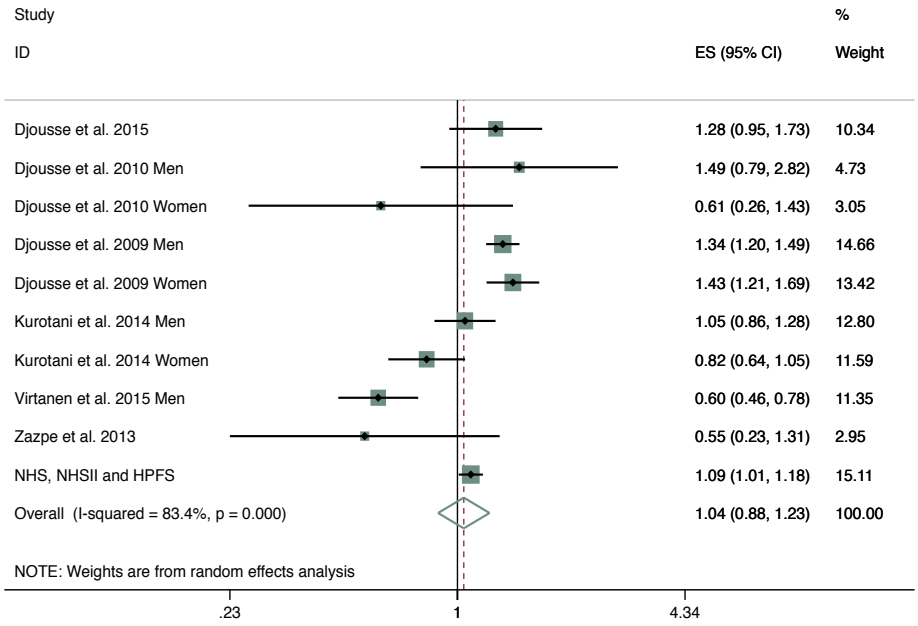


Figure 9. Risk of Type 2 Diabetes Associated with an Increase in Consumption of 1 egg/day using fixed (A) and random (B) effects meta-analysis. Weights of each of the studies are represented by the size of the square. Black diamonds represent the individual study effects and black lines represent the 95% confidence intervals. The overall effect estimate and 95% confidence interval is represented by the diamond and dotted line. Abbreviations: Nurses' Health Study (NHS), Nurses' Health Study II (NHSII), Health Professionals Follow-up Study (HPFS).

DISCUSSION

After adjustment for dietary and lifestyle factors we observed a modest association between higher egg intake and risk of T2D. In the meta-analysis of 9 cohorts, we found a similar association between egg intake of one egg per day and risk of T2D that was significant for the fixed effects meta-analysis, but not for random effects. We observed a significantly decreased risk of T2D when substituting eggs for legumes, low-fat dairy, nuts and whole grains, and a significantly increased risk of T2D when substituting eggs for red and processed meats. These results suggest that healthy alternatives to egg consumption include legumes, low-fat dairy, nuts and whole grains.

Findings from previously published cohort studies have been inconsistent, likely due to the differences in adjustment for confounders and follow-up time. The Japan Public Health Center-based prospective study, the Sun Project, the Jackson Heart Study and the Kuopio Ischaemic Heart Disease Risk Factor Study did not find an increased risk of T2D associated with egg intake after adjustment for potential confounders including dietary variables [19-21, 60]. While the Physician's Health Study, Women's Health Study and men in the Cardiovascular Health Study reported a significantly increased risk of T2D with higher egg consumption, it is possible that the associations seen in the Physician's Health and Cardiovascular Health Studies would have been attenuated if authors had been able to adjust for additional confounders such as diet, family history of T2D, and history of hypertension and high blood cholesterol [16, 59]. Recently, the upper limit for dietary cholesterol was not carried forward in the 2015 Dietary Guidelines Advisory Committee Report due to the lack of an appreciable relationship between dietary and blood cholesterol [3]. Although eggs are a major source of dietary cholesterol, containing about 200mg, they also provide a variety of other nutrients beneficial to health such as unsaturated fats, amino acids, and B-vitamins, potentially offsetting any harmful effects of dietary cholesterol [4]. In our cohorts, there was a slight positive association between dietary cholesterol and T2D, but there was no dose response relationship. In addition, adjustment for dietary cholesterol further attenuated the association between egg consumption and T2D, suggesting that the effect of egg intake is mediated by dietary cholesterol.

We observed a consistent association between egg consumption and T2D across subgroups in several stratified analyses by diabetes risk factors. However, we also observed significant interactions for hypertension, high blood cholesterol and statin use, which we cannot explain. Of note, we observed no significant changes in egg intake after diagnosis of hypertension, high blood cholesterol or beginning statin use; however, overall egg intake was lower in these participants. No previous publications have reported similar interactions; therefore, these results should be interpreted with caution and require further confirmation.

It is possible that the increased risk of T2D seen among participants with normal blood cholesterol is due to some additional unmeasured confounding among less healthy participants, obscuring the relationship between egg consumption and T2D seen among the healthy population. Indeed, the risk of T2D for an increase of 1 egg per day is attenuated in participants who report a higher number of unhealthy lifestyle factors such as low physical activity, BMI>25, family history of type 2 diabetes, hypertension, high blood cholesterol, angina, CABG and smoking.

We observed a large amount of heterogeneity in our meta-analysis. There was no one study that contributed a significant amount of heterogeneity, and stratification by number of cases, total number of participants, sex, country, length of follow-up and whether there were repeated measures of diet during follow-up could not explain the differences among studies. Although we see a significantly increased risk of T2D with egg intake in the fixed effects meta-analysis, the relationship is attenuated when random effects is used. Therefore, results from the meta-analysis should be interpreted cautiously.

These analyses provide several strengths including the large sample size, detailed and updated information on diet and lifestyle, and high rates of follow-up. Previously published cohort studies have lacked sufficient follow-up time or adjustment for dietary confounders. In the current analyses we were able to adjust for dietary factors related to egg intake such as bacon, red and processed meat, coffee, juice, sugar sweetened beverages, whole milk and refined carbohydrates. In addition, our analysis includes 16,867 cases, while the largest previous study, the Women's Health Study includes only 2,112 cases [59].

The collection of updated dietary information and use of cumulative average update allows us to account for differences in intake within people and limit random measurement error, while allowing for changes over time. Thus, these analyses also offer the ability to strengthen the meta-analysis of current literature.

There are limitations of the current analyses. There is a possibility of misclassification of dietary variables due to random measurement error possible with the FFQ. If present, this type of error would cause an underestimation of the association between egg intake and T2D. Dietary intake measured via the FFQ has been validated against weighed 7-day records and shows high de-attenuated correlations for egg intake (NHS=0.77, HPFS=0.80) [31]. In addition, the method for updating dietary information, cumulative average update, minimizes some of the possible measurement error due to the FFQ. Although our sample is less generalizable to other populations due to the participants being predominantly of European ancestry, the high education level in this population has likely enhanced the quality of the data collected. Due to the observational nature of this study we cannot infer causation and cannot eliminate the possibility that some unmeasured or residual confounding remains; however, this is limited by adjustment for a number of possible confounders including dietary and lifestyle factors.

Higher egg intake is associated with a small increased risk of T2D in the prospective cohort studies NHS, NHSII and HPFS. These results were quantitatively similar to a meta-analysis of the current literature, which also found a significant association with egg intake and risk of T2D using random effects, but not with fixed effects. In addition, our analyses suggest that healthy alternatives to egg intake include legumes, low-fat dairy, nuts and whole grains.

CONCLUSION

The prevention of chronic diseases such as T2D and CVD are of high importance. Many foods are associated with increased or decreased risks of these diseases, and it is important to understand these relationships to make food-based dietary recommendations. Specifically, for egg intake, the debate has lasted decades from the original proposal of the diet-heart hypothesis.

In chapter 1, we did not observe any association between egg intake and the risk of CVD in NHSI, NHSII or HPFS. However, we observed an increased risk of CVD among participants with T2D. We also found that replacing eggs with one serving of low fat dairy, whole grains or nuts resulted in a decreased risk of CVD, while replacing eggs with red and processed meats resulted in an increased risk of CVD.

In chapter 2, we observed a small positive association between egg intake and the risk of CVD mortality in the AARP study. It is possible that this increase may be driven by participants with T2D, evidenced by the increased risk of CVD mortality with egg intake seen among participants reporting T2D at baseline. When we combined previously published cohorts using meta-analysis we saw a slight positive association between increases of one egg per day and risk of CVD. This association was no longer present when we used a random-effects meta-analysis and may be due to unmeasured confounding in many of the studies.

In chapter 3, we found an increased risk of T2D with an increase in intake of one egg per day in the NHS, NHSII and HPFS cohorts. Replacement of eggs for legumes, low fat dairy, nuts and whole grains all resulted in a decreased risk of T2D, while replacement of eggs with red and processed meats resulted in an increased risk. These findings were quantitatively similar to our meta-analysis; however, results were not statistically significant using random effects. These meta-analysis results should be interpreted with caution due to the large amount of heterogeneity between the studies included.

In summary, it appears that there is no association between egg intake and risk of CVD among healthy individuals. However, individuals at higher risk for CVD, such as those with T2D, may consider reducing egg intake. In addition, there is a slight increased risk of T2D with increases in egg intake of one

egg per day. Further research is needed to confirm findings among people at higher risk for CVD and T2D. Although eggs can be included as part of a healthy diet in individuals at low risk for CVD and T2D, there are many healthy alternatives to egg intake, which include low fat dairy, whole grains, legumes and nuts.

REFERENCES

1. Krauss RM, Eckel RH, Howard B, Appel LJ, Daniels SR, Deckelbaum RJ, Erdman JW, Jr., Kris-Etherton P, Goldberg IJ, Kotchen TA, Lichtenstein AH, Mitch WE, Mullis R, Robinson K, Wylie-Rosett J, St Jeor S, Suttie J, Tribble DL and Bazzarre TL. AHA Dietary Guidelines: revision 2000: A statement for healthcare professionals from the Nutrition Committee of the American Heart Association. *Circulation*. 2000;102:2284-99.
2. Hu FB, Manson JE, Stampfer MJ, Colditz G, Liu S, Solomon CG and Willett WC. Diet, lifestyle, and the risk of type 2 diabetes mellitus in women. *The New England journal of medicine*. 2001;345:790-7.
3. Millen BEea. Scientific Report of the 2015 Dietary Guidelines Advisory Committee. 2015:3.
4. Hu FB, Stampfer MJ, Rimm EB, Manson JE, Ascherio A, Colditz GA, Rosner BA, Spiegelman D, Speizer FE, Sacks FM, Hennekens CH and Willett WC. A prospective study of egg consumption and risk of cardiovascular disease in men and women. *JAMA : the journal of the American Medical Association*. 1999;281:1387-94.
5. Hu FB, van Dam RM and Liu S. Diet and risk of Type II diabetes: the role of types of fat and carbohydrate. *Diabetologia*. 2001;44:805-17.
6. Willett WC. Dietary fats and coronary heart disease. *J Intern Med*. 2012;272:13-24.
7. Spence JD, Jenkins DJ and Davignon J. Egg yolk consumption and carotid plaque. *Atherosclerosis*. 2012;224:469-73.
8. Nakamura Y, Iso H, Kita Y, Ueshima H, Okada K, Konishi M, Inoue M and Tsugane S. Egg consumption, serum total cholesterol concentrations and coronary heart disease incidence: Japan Public Health Center-based prospective study. *Br J Nutr*. 2006;96:921-8.
9. Nakamura Y, Okamura T, Tamaki S, Kadowaki T, Hayakawa T, Kita Y, Okayama A and Ueshima H. Egg consumption, serum cholesterol, and cause-specific and all-cause mortality: the National Integrated Project for Prospective Observation of Non-communicable Disease and Its Trends in the Aged, 1980 (NIPPON DATA80). *Am J Clin Nutr*. 2004;80:58-63.

10. Qureshi AI, Suri FK, Ahmed S, Nasar A, Divani AA and Kirmani JF. Regular egg consumption does not increase the risk of stroke and cardiovascular diseases. *Med Sci Monit*. 2007;13:CR1-8.
11. Djousse L and Gaziano JM. Egg consumption in relation to cardiovascular disease and mortality: the Physicians' Health Study. *Am J Clin Nutr*. 2008;87:964-9.
12. Sauvaget C, Nagano J, Allen N, Grant EJ and Beral V. Intake of animal products and stroke mortality in the Hiroshima/Nagasaki Life Span Study. *Int J Epidemiol*. 2003;32:536-43.
13. Scrafford CG, Tran NL, Barraj LM and Mink PJ. Egg consumption and CHD and stroke mortality: a prospective study of US adults. *Public Health Nutr*. 2011;14:261-70.
14. Bernstein AM, Pan A, Rexrode KM, Stampfer M, Hu FB, Mozaffarian D and Willett WC. Dietary protein sources and the risk of stroke in men and women. *Stroke*. 2012;43:637-44.
15. Djousse L, Gaziano JM, Buring JE and Lee IM. Egg consumption and risk of type 2 diabetes in men and women: Response to Citrome and Holt. *Diabetes Care*. 2009;32:e74.
16. Djousse L, Kamineni A, Nelson TL, Carnethon M, Mozaffarian D, Siscovick D and Mukamal KJ. Egg consumption and risk of type 2 diabetes in older adults. *Am J Clin Nutr*. 2010;92:422-7.
17. Vang A, Singh PN, Lee JW, Haddad EH and Brinegar CH. Meats, processed meats, obesity, weight gain and occurrence of diabetes among adults: findings from Adventist Health Studies. *Annals of nutrition & metabolism*. 2008;52:96-104.
18. Zazpe I, Beunza JJ, Bes-Rastrollo M, Basterra-Gortari FJ, Mari-Sanchis A and Martinez-Gonzalez MA. Egg consumption and risk of type 2 diabetes in a Mediterranean cohort; the sun project. *Nutricion hospitalaria*. 2013;28:105-11.
19. Djousse L, Petrone AB, Hickson DA, Talegawkar SA, Dubbert PM, Taylor H and Tucker KL. Egg consumption and risk of type 2 diabetes among African Americans: The Jackson Heart Study. *Clinical nutrition*. 2015.
20. Kurotani K, Nanri A, Goto A, Mizoue T, Noda M, Oba S, Sawada N, Tsugane S and Japan Public Health Center-based Prospective Study G. Cholesterol and egg intakes and the risk of type 2 diabetes: the Japan Public Health Center-based Prospective Study. *Br J Nutr*. 2014;112:1636-43.

21. Virtanen JK, Mursu J, Tuomainen TP, Virtanen HE and Voutilainen S. Egg consumption and risk of incident type 2 diabetes in men: the Kuopio Ischaemic Heart Disease Risk Factor Study. *Am J Clin Nutr*. 2015;101:1088-96.

22. Hao M, Head WS, Gunawardana SC, Hasty AH and Piston DW. Direct effect of cholesterol on insulin secretion: a novel mechanism for pancreatic beta-cell dysfunction. *Diabetes*. 2007;56:2328-38.

23. Kruit JK, Kremer PH, Dai L, Tang R, Ruddle P, de Haan W, Brunham LR, Verchere CB and Hayden MR. Cholesterol efflux via ATP-binding cassette transporter A1 (ABCA1) and cholesterol uptake via the LDL receptor influences cholesterol-induced impairment of beta cell function in mice. *Diabetologia*. 2010;53:1110-9.

24. Kung HC, Hoyert DL, Xu J and Murphy SL. Deaths: final data for 2005. *Natl Vital Stat Rep*. 2008;56:1-120.

25. Shin JY, Xun P and He K. Egg consumption and risk of cardiovascular disease: Meta-analysis. *Diabetes*. 2012;61:A379-A380.

26. Barona J and Fernandez ML. Dietary Cholesterol Affects Plasma Lipid Levels, the Intravascular Processing of Lipoproteins and Reverse Cholesterol Transport without Increasing the Risk for Heart Disease. *Nutrients*. 2012;4:1015-25.

27. Rong Y, Chen L, Zhu T, Song Y, Yu M, Shan Z, Sands A, Hu FB and Liu L. Egg consumption and risk of coronary heart disease and stroke: dose-response meta-analysis of prospective cohort studies. *Bmj*. 2013;346:e8539.

28. Colditz GA, Manson JE and Hankinson SE. The Nurses' Health Study: 20-year contribution to the understanding of health among women. *Journal of women's health / the official publication of the Society for the Advancement of Women's Health Research*. 1997;6:49-62.

29. Rimm EB, Giovannucci EL, Willett WC, Colditz GA, Ascherio A, Rosner B and Stampfer MJ. Prospective study of alcohol consumption and risk of coronary disease in men. *Lancet*. 1991;338:464-8.

30. Solomon CG, Willett WC, Carey VJ, Rich-Edwards J, Hunter DJ, Colditz GA, Stampfer MJ, Speizer FE, Spiegelman D and Manson JE. A prospective study of pregravid determinants of gestational diabetes mellitus. *JAMA : the journal of the American Medical Association*. 1997;278:1078-83.
31. Willett WC. Reproducibility and Validity of Food-Frequency Questionnaires *Nutritional Epidemiology.* Second Edition ed. New York: Oxford University Press; 1998.
32. Salvini S, Hunter DJ, Sampson L, Stampfer MJ, Colditz GA, Rosner B and Willett WC. Food-based validation of a dietary questionnaire: the effects of week-to-week variation in food consumption. *Int J Epidemiol*. 1989;18:858-67.
33. Feskanich D, Rimm EB, Giovannucci EL, Colditz GA, Stampfer MJ, Litin LB and Willett WC. Reproducibility and validity of food intake measurements from a semiquantitative food frequency questionnaire. *J Am Diet Assoc*. 1993;93:790-6.
34. Rose GAB, H.; Gillum, R.F.; Prineas, R.J. Cardiovascular Survey Methods. 1982.
35. Walker AE, Robins M and Weinfeld FD. The National Survey of Stroke. Clinical findings. *Stroke*. 1981;12:I13-44.
36. Bamford J, Sandercock P, Dennis M, Burn J and Warlow C. Classification and natural history of clinically identifiable subtypes of cerebral infarction. *Lancet*. 1991;337:1521-6.
37. Hu FB, Stampfer MJ, Rimm E, Ascherio A, Rosner BA, Spiegelman D and Willett WC. Dietary fat and coronary heart disease: a comparison of approaches for adjusting for total energy intake and modeling repeated dietary measurements. *Am J Epidemiol*. 1999;149:531-40.
38. Greenland S and Longnecker MP. Methods for trend estimation from summarized dose-response data, with applications to meta-analysis. *Am J Epidemiol*. 1992;135:1301-9.
39. Shekelle RB, Stamler J, Paul O, Shryock AM, Liu S and Lepper M. Dietary lipids and serum cholesterol level: change in diet confounds the cross-sectional association. *Am J Epidemiol*. 1982;115:506-14.

40. Bernstein AM, Sun Q, Hu FB, Stampfer MJ, Manson JE and Willett WC. Major dietary protein sources and risk of coronary heart disease in women. *Circulation*. 2010;122:876-83.
41. Djousse L and Gaziano JM. Egg consumption and risk of heart failure in the Physicians' Health Study. *Circulation*. 2008;117:512-6.
42. Zazpe I, Beunza JJ, Bes-Rastrollo M, Warnberg J, de la Fuente-Arrillaga C, Benito S, Vazquez Z and Martinez-Gonzalez MA. Egg consumption and risk of cardiovascular disease in the SUN Project. *European journal of clinical nutrition*. 2011;65:676-82.
43. Smahelova A, Hyspler R and Haas T. Relation of cholesterol metabolism and non-cholesterol sterols to insulin resistance. *Physiological research / Academia Scientiarum Bohemoslovaca*. 2007;56:749-55.
44. Howell WH, McNamara DJ, Tosca MA, Smith BT and Gaines JA. Plasma lipid and lipoprotein responses to dietary fat and cholesterol: a meta-analysis. *Am J Clin Nutr*. 1997;65:1747-64.
45. McGill HC, Jr. The relationship of dietary cholesterol to serum cholesterol concentration and to atherosclerosis in man. *Am J Clin Nutr*. 1979;32:2664-702.
46. Eckel RH, Jakicic JM, Ard JD, de Jesus JM, Houston Miller N, Hubbard VS, Lee IM, Lichtenstein AH, Loria CM, Millen BE, Nonas CA, Sacks FM, Smith SC, Jr., Svetkey LP, Wadden TA, Yanovski SZ and American College of Cardiology/American Heart Association Task Force on Practice G. 2013 AHA/ACC guideline on lifestyle management to reduce cardiovascular risk: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *J Am Coll Cardiol*. 2014;63:2960-84.
47. Tang WH, Wang Z, Levison BS, Koeth RA, Britt EB, Fu X, Wu Y and Hazen SL. Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. *The New England journal of medicine*. 2013;368:1575-84.
48. Sands ALL, Y.; Stampfer, M.J.; Sacks, F.M.; Rosner, B.; Willett, W.C.; Hu, F.B. Egg Consumption and Risk of Cardiovascular Disease: Findings from Three Large Prospective US Cohort Studies of Men and Women. *Unpublished*.

49. Schatzkin A, Subar AF, Thompson FE, Harlan LC, Tangrea J, Hollenbeck AR, Hurwitz PE, Coyle L, Schussler N, Michaud DS, Freedman LS, Brown CC, Midthune D and Kipnis V. Design and serendipity in establishing a large cohort with wide dietary intake distributions : the National Institutes of Health-American Association of Retired Persons Diet and Health Study. *Am J Epidemiol*. 2001;154:1119-25.
50. Thompson FE, Kipnis V, Midthune D, Freedman LS, Carroll RJ, Subar AF, Brown CC, Butcher MS, Mouw T, Leitzmann M and Schatzkin A. Performance of a food-frequency questionnaire in the US NIH-AARP (National Institutes of Health-American Association of Retired Persons) Diet and Health Study. *Public Health Nutr*. 2008;11:183-95.
51. Box GEPC, D.R. An analysis of transformations. *Journal of the Royal Statistical Style*. 1964;Series B:211-252.
52. Orsini NB, R.; Greenland, S. . Generalized least squares for trend estimation of summarized dose-response data. *Stata Journal*. 2006;6:40-57.
53. Fielding CJ, Castro GR, Donner C, Fielding PE and Reaven GM. Distribution of apolipoprotein E in the plasma of insulin-dependent and noninsulin-dependent diabetics and its relation to cholesterol net transport. *Journal of lipid research*. 1986;27:1052-61.
54. Venkatesan S, Imrie H, Read S and Halliday D. Apo C subclasses from non-insulin-dependent diabetic patients--a quantitative comparison with control subjects. *Biochemical Society transactions*. 1995;23:278S.
55. Borggreve SE, De Vries R and Dullaart RP. Alterations in high-density lipoprotein metabolism and reverse cholesterol transport in insulin resistance and type 2 diabetes mellitus: role of lipolytic enzymes, lecithin:cholesterol acyltransferase and lipid transfer proteins. *Eur J Clin Invest*. 2003;33:1051-69.
56. Howard BV. Insulin resistance and lipid metabolism. *The American journal of cardiology*. 1999;84:28J-32J.

57. Ford ES and Capewell S. Trends in total and low-density lipoprotein cholesterol among U.S. adults: contributions of changes in dietary fat intake and use of cholesterol-lowering medications. *PloS one*. 2013;8:e65228.

58. Schwab U, Lauritzen L, Tholstrup T, Haldorssoni T, Riserus U, Uusitupa M and Becker W. Effect of the amount and type of dietary fat on cardiometabolic risk factors and risk of developing type 2 diabetes, cardiovascular diseases, and cancer: a systematic review. *Food & nutrition research*. 2014;58.

59. Djousse L, Gaziano JM, Buring JE and Lee IM. Egg consumption and risk of type 2 diabetes in men and women. *Diabetes Care*. 2009;32:295-300.

60. Zazpe I, Beunza JJ, Bes-Rastrollo M, Basterra-Gortari FJ, Mari-Sanchis A, Martinez-Gonzalez MA and Investigators SUNP. Egg consumption and risk of type 2 diabetes in a Mediterranean cohort; the sun project. *Nutricion hospitalaria*. 2013;28:105-11.

61. Li Y, Zhou C, Zhou X and Li L. Egg consumption and risk of cardiovascular diseases and diabetes: a meta-analysis. *Atherosclerosis*. 2013;229:524-30.

62. Manson JE, Rimm EB, Stampfer MJ, Colditz GA, Willett WC, Krolewski AS, Rosner B, Hennekens CH and Speizer FE. Physical activity and incidence of non-insulin-dependent diabetes mellitus in women. *Lancet*. 1991;338:774-8.

63. Hu FB, Leitzmann MF, Stampfer MJ, Colditz GA, Willett WC and Rimm EB. Physical activity and television watching in relation to risk for type 2 diabetes mellitus in men. *Archives of internal medicine*. 2001;161:1542-8.

64. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care*. 1997;20:1183-97.